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Influence of duodenal secretions and its components on release and activities of human brush-border enzymes

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The in vitro effects of human duodenal secretions and various combinations of its components on activity and release of enzymes from the human brush border were examined. Sucrase retained activity for 90 min in duodenal secretions, and maltase was almost as stable; lactase lost activity rapidly and alkaline phosphatase was of intermediate stability. Inactivation of lactase could only be partly (50%) attributed to luminal proteases, bile salts and phospholipids played no role. Rate of release of an enzyme from the brush border bore no relationship to its rate of inactivation. When individual proteases were studied, elastase was the most potent for releasing disaccharidases from the brush border; trypsin was ineffective alone but augmented the effect of elastase. Sucrase and maltase were activated by proteolytic release, but activation was abolished by simultaneous exposure of brush borders to bile salts. Lactase was released and rapidly inactivated by proteinases, while alkaline phosphatase appeared to be inactivated without significant release. These results show that there are significant interactions between luminal factors which have been inapparent when studying them in isolation. Loss of functionally useful enzyme does not follow release of sucrase or maltase from the brush border into the lumen but does follow release of lactase. Study of the susceptibility of lactase to inactivation by luminal factors in the various forms of lactose intolerance is warranted.

Introduction

Studies in a number of species have indicated that the digestive function of the intestinal brush border is influenced by multiple environmental factors. One determinant of the mass of brush-border enzymes is the continued replication of cells in the crypt followed by migration onto the villus where cells mature and synthesise large amounts of these enzymes to maintain adequate levels [1]. Conditions in which there is a reduction of villus cell mass, e.g., celiac disease, result in a reduction in mucosal brush-border enzyme activities [2,3].

Brush-border enzymes are, however, turned over more rapidly than cells or mucosal proteins in general [4,5]. Their activities are more directly affected by a variety of environmental influences acting from the lumen. The majority of nutrients for the villus cell come from the gut lumen [1]. Dietary substrates can induce the corresponding enzyme, e.g., sucrose feeding induces sucrase [6,7]. Other luminal factors, such as some of the pancreatic proteinases and bile salts, have been shown to be capable of inactivating or releasing certain enzymes from the brush border [8–10]. But the interaction of these various factors, which coexist in the intestinal lumen, has not been studied except in the case of the brush-border enzyme enterokinase. Its activation of trypsinogen is facilitated by bile salts [9,11].

Few studies have examined the way in which enzymes of the human intestinal brush border react to the various potentially injurious components present in intestinal contents. Certain pancreatic and bacterial proteinases have been shown to release brush-border enzymes in a soluble form [12–16], but the resultant effect on enzyme activity is not clear, especially when these agents are combined with other luminal factors. Membrane-perturbing substances, such as bile salts and dietary and biliary phospholipids, might modify these actions of the proteinases.

The purpose of this study was to examine the effect of human intestinal luminal contents and its various components on selected enzymes of the human brush border, paying particular attention to the effects of combinations of these components. Both the effects on enzyme activity and release from the membrane were

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studied. Part of this work has been previously published in abstract form [17].

Materials and Methods

Intestinal mucosa

Human small intestine (distal jejunum, Caucasian subjects) was obtained from operative specimens obtained during the course of surgical resection for jejunoileal bypass (two patients) or subacute obstruction caused by adhesions (two patients). The comparability of human duodenum, jejunum and ileum in enzymatic release experiments has already been demonstrated [13]. Light microscopy of the segments studied confirmed normal villus architecture and morphology. Specific activity of mucosal sucrase and maltase fell within the upper half of the laboratory's normal range (unpublished) for these enzymes in normal proximal jejunal tissue obtained by capsule biopsy. Segments of intestine were placed on ice and transported to the laboratory where the mucosa was scraped from submucosa using glass slides, snap frozen in liquid nitrogen and stored at $-70\,^{\circ}$C.

Preparation of brush borders

Brush-border fragments were prepared from thawed mucosal scrapings using a variation of the method of Schmitz et al. [18]. Nine volumes of ice-cold 10 mmol/l Tris-HCl, 0.1 mmol/l $MgCl_2$, 50 mmol/l mannitol (buffer A) were added to 5–10 g of scrapings which were dispersed by a few strokes in a Potter-Elvehjeim homogeniser with a loose-fitting teflon pestle rotating at 75% of maximum speed (approx. 1500 rpm). Additional ice-cold buffer A was then added to give a 4% suspension. This suspension was homogenised in a Waring Commercial blender at speed '4' for 30 s and filtered through a nylon stocking. The filtrate was stirred at $4\,^{\circ}$C, $CaCl_2$ was added to 20 mmol/l and stirring continued for 10 min, then centrifuged at $3000 \times g_{max}$ for 10 min. The supernatant fraction (S_1) was retained and the pellet (P_1) resuspended in 40 ml ice-cold buffer A. It was rehomogenised with five strokes of the Potter-Elvehjeim homogeniser, reprecipitated as above with 20 mmol/l $CaCl_2$ and recentrifuged. The resulting pellet was discarded, while the supernatant fraction (S_1') was pooled with S_1 and centrifuged at $27\,000 \times g_{max}$ for 30 min. The final pellet (P_2) contained brush-border fragments and was resuspended in 14.3 mmol/l phosphate-buffered saline (pH 7.4) ('incubation buffer') at 1 mg membrane protein per ml.

Enzyme assays

Prior to enzyme assay, Triton X-100 was added to 0.1% (v/v) to all membrane suspensions. Disaccharidases, maltase (α-D-glucoside glucohydrolase, EC 3.2.1.20), lactase (β-D-galactoside galactohydrolase, EC 3.2.1.23) and sucrase (sucrose α-glucohydrolase, EC 3.2.1.48), were assayed as described [19] except that the samples were boiled for 60 s immediately before addition of the TGO reagent. Protein was estimated by the method of Lowry et al. [20] using crystalline bovine serum albumin as standard. Alkaline phosphatase (EC 3.1.3.1) was assayed as described previously [21]. Enzyme activities are expressed as units (μmole substrate released per min at $37\,^{\circ}$C) per mg protein. Trypsin and elastase in duodenal secretions were measured in a fibrinolytic assay system [22] using specific enzyme inhibitors [23] to allow estimation of the proportion of total proteolytic (i.e., fibrinolytic) activity attributable to each enzyme. Commercially available enzymes were similarly assayed and amounts comparable in activity to the duodenal secretions, used for the incubation experiments described below.

Materials

Laboratory reagents of reagent grade were obtained from Sigma Chemical Co., St. Louis, MO unless otherwise stated. Special materials used were trypsin (EC 3.4.4.4) (15 500 BAEE units per mg protein; porcine type IX), elastase (EC 3.4.21.11) (120 units per mg protein, porcine type III), glycochenodeoxycholic acid (sodium salt), glycocholic acid (sodium salt), L-α-phosphatidyl choline (egg, type I-EH), and L-α-phosphatidyl/choline (egg, type I). Secretin and cholecystokinin were obtained from Boots Australia P/L.

Collection of human duodenal secretions

A fasting healthy male volunteer underwent intubation of the distal duodenum under fluoroscopic control. Luminal secretions were collected for 1 h after an intravenous injection of 100 U secretin and 100 U cholecystokinin. pH of the aspirated fluid was 7.5. When it was necessary to inhibit serine proteases, phenylmethylsulphonyl fluoride (PMSF) was added to 0.1 mmol/l and aprotonin to 1 mmol/l. The 'proteolytic' fraction of duodenal secretions was extracted by precipitation of protein in ethanol (85%) at $-20\,^{\circ}$C [24]. The precipitated proteins were then redissolved in phosphate-buffered saline. Greater than 95% extraction of bile salts into the ethanolic phase was confirmed by comparing pre- and post-treatment spectrophotometric absorbances (A_{310}, cholic acid; A_{350}, deoxycholate [25]).

Brush-border incubation experiments

1-ml aliquots of resuspended, freshly prepared brush border were incubated in capped test tubes at $37\,^{\circ}$C in an orbital shaking waterbath (100 cycles per minute; model OW1412, Paton Industries P/L, South Australia), with an equal volume of either duodenal secretions, protease extract of duodenal secretions, or incubation buffer containing various combinations of pancreatic enzymes, bile salts and phospholipids. Control incuba-

tions contained brush border in incubation buffer only. Aliquots were removed at selected time points, immediately diluted 1:4 with ice-cold incubation buffer containing 0.1 mmol/l PMSF and 1 mmol/l aprotonin, and centrifuged at 27000 × g_{max} for 30 min. Centrifugation at this speed does separate truly soluble from membrane-bound enzymes [24]. The resultant pellet was resuspended in ice-cold buffer A to give a total volume equal to that of the supernatant fraction. Both supernatant fraction and resuspended pellet were stored at −20 °C until assayed. Enzymes were stable during the period of storage (up to two weeks). The proportion of enzyme present in the supernatant fraction was expressed as a proportion of total activity at each time point and was referred to as the amount 'solubilised' or 'released'. Changes in total enzyme activity during incubation, i.e., 'residual activity', were determined from the sum of pellet and supernatant activities at each time point, and were expressed as a percentage of the zero-time value. A 'blank' tube containing duodenal secretions but no brush borders, was included for each incubation so that the small amount of enzyme activity present could be subtracted from activities in the test incubations. Aliquots of incubated brush borders were diluted 1 in 10 during assay of alkaline phosphatase to avoid inhibition of the enzyme.

Electron microscopy

Purified brush borders were pelletted in cellulose nitrate tubes, fixed in 2.5% glutaraldehyde and embedded in Epon before sectioning for examination.

Results

Isolation of brush borders

The method used gave yields of 28–37% of sucrase relative to the starting homogenate prepared from the four jejunums studied. The purification factor for sucrase averaged 11.0 (range 9–12.7) and for alkaline phosphatase averaged 9.1 (range 8–12). Electron micrographs (not shown) revealed vesicles which varied in shape and size, and membrane fragments which did not form closed vesicles. Knob-like particles were seen on the outside of the vesicles suggesting that they were right-side out. Assay of sucrase and alkaline phosphatase in brush borders suspended in incubation buffer containing 1% Triton X-100 showed 7–9% activation of sucrase activity relative to buffer without detergent, thus confirming the right-side out orientation of the vesicles.

Incubation of brush borders

Each enzyme showed an individual pattern over time with respect to its solubilisation and/or inactivation by luminal secretions. Variations in these responses between preparations from different intestines were never greater than 20%.

Incubation in untreated secretions

Fig. 1A shows the effects on total activity (i.e., supernatant plus brush-border pellet) of the various brush-border enzymes brought about by in vitro exposure of brush-border fragments to untreated duodenal secretions at 37 °C for 90 min. Sucrase and maltase were the most stable and retained at least 80% activity after a 60 min exposure. Lactase and alkaline phosphatase lost activity rapidly with less than 25% activity remaining at 60 min. Incubation of brush borders in buffer (Fig. 1B) was accompanied by a small loss in activity for the disaccharidases; activities remaining at 60 min were sucrase (91%), maltase (92%), and lactase (93%). Alkaline phosphatase activity had fallen by 45% at 60 min

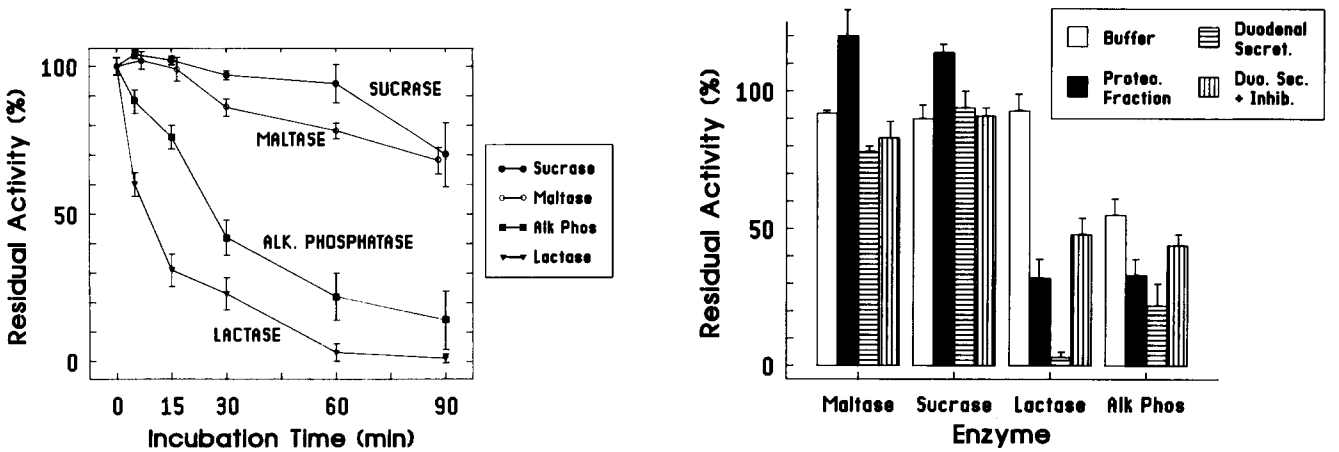

Fig. 1.(A) Effect on enzyme activities of incubation of human brush borders in duodenal secretions at 37 °C, for up to 90 min. Enzyme activities are expressed relative to zero-time levels and represent residual activity in brush-border pellet plus supernatant fraction. Vertical bars represent S.E. for studies in brush borders from four separate jejunal specimens. (B) Effect on enzyme activities of incubation of brush borders in human duodenal secretions (Duodenal Secret.), buffer, duodenal secretions plus protease inhibitors (Duo. Sec. + Inhib.) or proteolytic fraction (Proteo. Fraction) of duodenal secretions at 37 °C for 60 min. Enzyme activities are expressed relative to zero-time levels. Vertical bars represent the S.E. for four separate experiments.

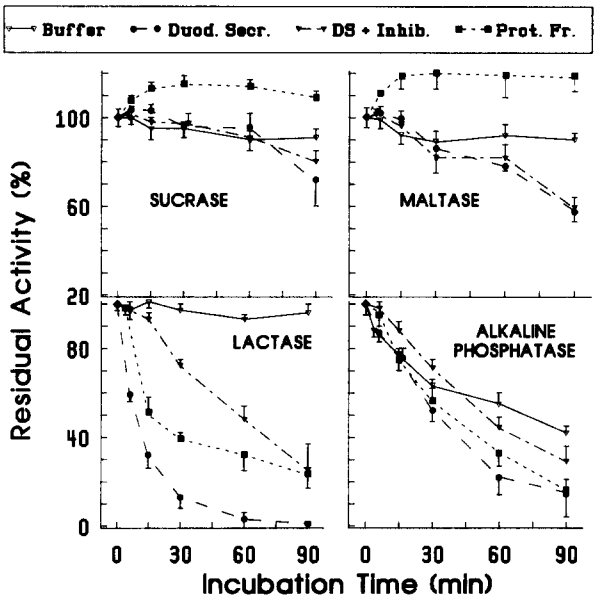

Fig. 2. Time course of changes in enzyme activities during incubation of brush borders in buffer, duodenal secretions (Duod. Secr.), duodenal secretions plus proteinase-inhibitors (DS + Inhib.) and the proteolytic fraction (Prot. Fr.) of duodenal secretions. Vertical bars show S.E. of four separate experiments.

in buffer, but this compared to an 80% loss of activity in duodenal secretions.

Incubation in treated duodenal secretions

To determine which elements of duodenal secretions were responsible for the loss of enzyme activity, secretions were treated in various ways. Addition of proteinase inhibitors (see Methods) reduced elastase activity from 11.8 mg equivalent elastase per ml to undetectable (< 0.4 mg/ml). Extraction of bile salts and phospholipids by treatment with ethanol (see Methods) resulted in 85% recovery of elastase and trypsin activity (while bile salts were undetectable) in the precipitated protein which readily redissolved in the incubation buffer (the 'proteolytic fraction'). Brush borders were exposed to these treated fractions and the effects are shown in Figs. 1B and 2.

The relatively small losses of activity of maltase and sucrase exposed to duodenal secretions were not significantly affected by addition of protease inhibitors. When brush borders were exposed to the proteolytic fraction, total activity of both enzymes increased significantly; rises were 30.4% for maltase and 26.7% for sucrase relative to control incubations in buffer.

Responses of lactase and alkaline phosphatase were quite different. The almost complete loss of lactase activity in duodenal juice, compared to the 70% loss when exposed to the proteolytic fraction not containing lipophilic compounds. Proteolytic inhibitors reversed the duodenal secretion-related loss of activity by 50%, but not 100%. Alkaline phosphatase was inherently unstable under all conditions, but activity was lost most quickly in fractions containing active proteolytic enzymes, and reversed by 69% relative to buffer control when proteinase inhibitors were added to duodenal secretions.

Examination of the time-course of events (Fig. 2) revealed that, in situations where activity was lost, it became apparent as early as 5–15 min and progressed with time. Activation of sucrase and maltase were maximal at 15 min.

Incubation of specific factors

In order to clarify which specific factors in duodenal secretions were responsible for loss of enzyme activity, brush borders were exposed to various agents and activities followed over 90 min. Results of 60 min exposure on enzyme activity are shown in Table I. Sucrase and maltase showed 18% and 27% activation, respectively, with elastase (relative to buffer), but not with trypsin. The presence of phospholipids or bile salts prevented activation of the enzyme, but in themselves did not lead to loss of activity.

Lactase lost 86% activity in duodenal secretions, compared to buffer, but less in the presence of elastase (33%) and none in the presence of trypsin. Neither phospholipid nor bile salts altered this effect of elastase. Alkaline phosphatase was again unstable under all conditions, but its activity was most affected when proteolytic enzymes, especially elastase, were present.

TABLE I

Effects of various components of duodenal secretions on brush-border enzyme activities

Brush borders were incubated in untreated duodenal secretions or incubation buffer containing additives at 37 °C. Results are shown for 60 min incubation.

Component	(% activity remaining at 60 min)			
	sucrase	maltase	lactase	alkaline phosphatase
Duodenal secretions	85 ± 4 [a]	83 ± 4	13 ± 5	52 ± 5
Bile salts [b]	97 ± 4	91 ± 6	103 ± 6	69 ± 8
Trypsin [c]/elastase [d]/ bile salts	99 ± 3	103 ± 8	79 ± 7	–
Trypsin	99 ± 6	97 ± 3	99 ± 5	63 ± 7
Elastase	112 ± 6	119 ± 24	77 ± 4	53 ± 4
Phospholipids [e]/ elastase	104 ± 3	100 ± 9	78 ± 3	61 ± 3
Phospholipids [e]	104 ± 3	100 ± 3	98 ± 4	69 ± 3
Buffer control	95 ± 3	94 ± 2	91 ± 7	68 ± 8

[a] ± S.E. derived from brush borders of four patients.
[b] Glycocholic (15 mmol/l) and glycochenodeoxycholic acids (10 mmol/l); approximate concentrations in hepatic bile [26] and approximately equal to the total concentrations in duodenal secretions as determined by spectrometry (see Methods).
[c] Trypsin 27.5 mg/ml.
[d] Elastase 11.8 mg/ml.
[e] Lecithin 0.2 mg/ml, lysolecithin 0.1 mg/ml; approx. one-tenth of concentration in bile [26].

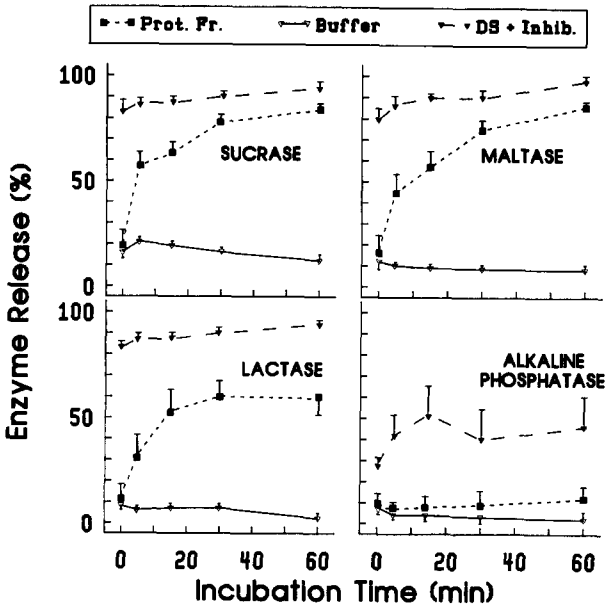

Fig. 3. Rate of release of brush-border enzymes from the brush border when incubated in duodenal secretions containing proteinase inhibitors (DS + Inhib.), the proteolytic fraction of duodenal secretions (Prot. Fr.) or buffer. Vertical bars show S.E. of four separate experiments.

Maximal degradation of brush-border enzymes was always seen in duodenal secretions. Even combinations of proteolytic enzymes with bile salts or phospholipids failed to fully reproduce the effects of duodenal secretions.

Release of brush-border enzymes

Fig. 3 shows the time course of solubilisation of enzymes over 60 min following exposure of brush borders to duodenal secretions containing proteinase inhibitors or to the proteolytic fraction. Solubilisation of the disaccharidases was very rapid in the duodenal secretions containing proteinase inhibitors, with 80% being solubilised in the first 5 min. Solubilisation proceeded more slowly in the proteolytic fraction, which contained minimal bile salts (see above), and did not reach maximal at 60–80% until 30–60 min. Only 50% of alkaline phosphatase activity could be solubilised by duodenal secretions containing inhibitors, and solubilisation proceeded very slowly in the proteolytic fraction.

When brush borders were exposed to untreated duodenal secretions (Fig. 4A) release of all enzymes was rapid. Lactase and alkaline phosphatase were rapidly inactivated in duodenal secretions, which makes quantitation of release at the later time points inexact.

When brush borders were exposed to the proteolytic fraction, the distribution of enzymes between supernatant fraction and pellet (Fig. 4B) was quite different from exposure to duodenal secretions. Enzymes were released much more slowly. Supernatant maltase and sucrase were stable over the duration of study after initial activation; supernatant lactase lost activity while only small amounts of active alkaline phosphatase appeared in the supernatant fraction.

The release from the brush border of each enzyme was restudied in the presence of individual proteolytic enzymes, bile salts, and various combinations of these. Fig. 5 shows that the initial rapid solubilisation of all enzymes seen in duodenal secretions (Figs. 3 and 4) was due to bile salts. Trypsin, at the concentration chosen (equivalent to duodenal secretions), was relatively ineffective in releasing any enzyme. Elastase led to progressive release of the disaccharidases. Combination of

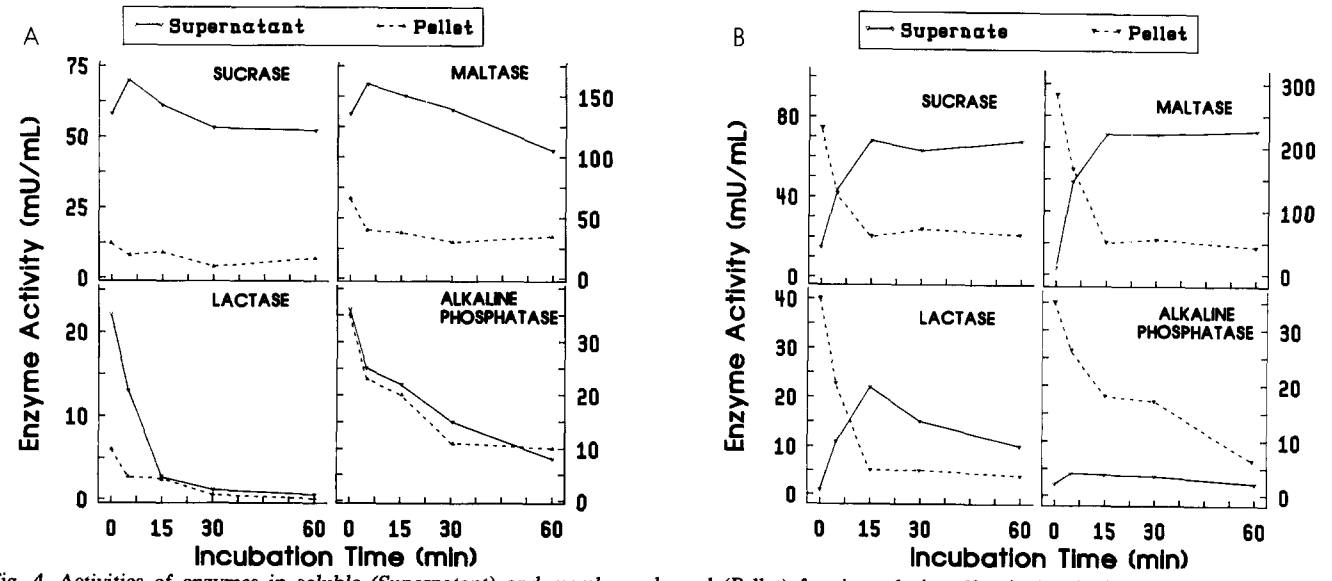

Fig. 4. Activities of enzymes in soluble (Supernatant) and membrane-bound (Pellet) fractions during 60 min incubation of brush borders in duodenal secretions (A), and in the proteolytic fraction of duodenal secretions (B). Each experiment is typical of all four; brush borders studied in A and B were from different subjects.

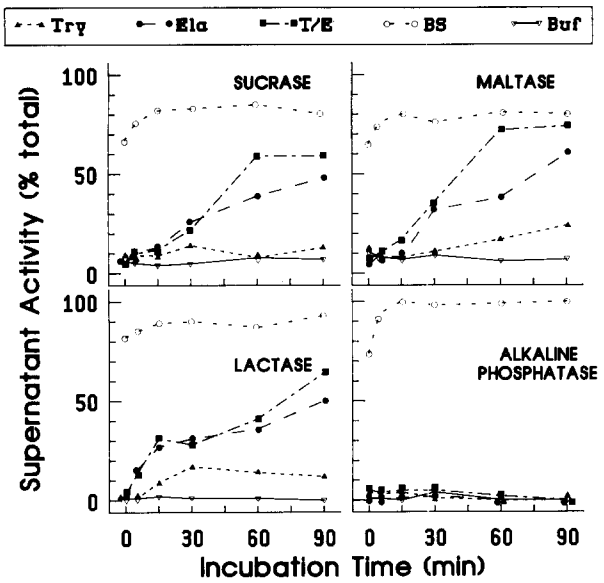

Fig. 5. Time course of release of enzymes into the supernatant fraction when brush borders were incubated in trypsin (Try), elastase (Ela), trypsin and elastase (T/E), bile salts (BS), and buffer (Buf). Release is expressed as percent of activity in the supernatant fraction at each time-point. See Table I for concentrations of additives. Data represent a typical experiment for the four jejunums studied.

TABLE II

Relationship between enzyme release and inactivation after incubations in treated or untreated duodenal secretions

Brush-border enzymes were incubated in untreated or treated duodenal secretions at 37 °C. Results are shown for 5 and 60 min time-points and expressed relative to the zero-time value. A negative value means that activation occurred.

Incubation conditions	Sucrase	Maltase	Lactase	Alkaline Phosphatase
Duodenal secretions				
5-min incubation				
% inactivation	−4±2 [a]	−2± 3	42± 4	12± 4
% released	89±3	89± 3	78± 9	41± 9
60-min incubation				
% inactivation	6±7	22± 2	97± 3	88± 8
% released	86±4	75± 7	inactive [b]	27±10
Duodenal secretions plus proteinase inhibitors				
5-min incubation				
% inactivation	−2±2	−2± 3	2± 4	2± 6
% released	87±3	88± 4	86± 3	41±11
6-min incubation				
% inactivation	10±3	18± 6	52± 6	56± 4
% released	93±4	92± 5	91± 4	45± 6
Proteolytic extract				
5-min incubation				
% inactivation	−9±2	−12± 1	2± 3	4± 6
% released	57±4	45±15	32±15	9± 2
60-min incubation				
% inactivation	−14±3	−19± 9	68± 7	67± 6
% released	82±7	83± 7	60±15	13± 4

[a] ± S.E. derived from brush borders of four patients.
[b] The degree of inactivation made calculation of release inexact.

trypsin with elastase augmented the elastase effect for each disaccharidase and in each brush-border preparation studied. Treatment by trypsin and/or elastase did not release active alkaline phosphatase into the supernatant fraction, although alkaline phosphatase activity fell to 25% of original over the 60 min (data not shown).

Relationship between release and inactivation

Table II shows the relationship between release of enzymes from the brush border and total enzyme activity after 5 min and 60 min incubation in vitro. Despite rapid release of substantial amounts of sucrase or maltase by either duodenal secretions, with or without protease inhibitors, or the proteolytic fraction, these enzymes were only slowly inactivated. Indeed, in the proteolytic extract, release was associated with prolonged augmentation of activity. The more rapid inactivation of lactase and alkaline phosphatase occurred in association with rapid appearance of active enzyme in the supernatant fraction when bile salts were present (duodenal secretions with or without protease inhibitors). Release was slower and less complete in proteolytic extract, with no more than 13% of alkaline phosphatase activity appearing in the supernatant fraction even though there was a 67% loss of activity.

Discussion

Studies in various species have indicated that in vivo turnover or in vitro release of brush-border enzymes by specific pancreatic proteinases, is different for each enzyme [27,28,9,29]. The present study confirms that these agents have parallel, but not identical effects on human brush-border enzymes. In addition, they show that there are significant interactions between the various membrane-perturbing agents, and that removal of an enzyme from the brush border is not necessarily synonymous with loss of hydrolytically useful enzyme, as has sometimes been considered to be the case [14].

When human brush borders were exposed to duodenal secretions or its components, sucrase was the most stable of the enzymes studied. The proteolytic fraction of duodenal secretions actually led to an increase in activity. Elastase and not trypsin was responsible for this effect, but addition of bile salts largely prevented activation. Thus, solubilisation itself is not responsible for activation. Perhaps proteolytic cleavage of the membrane-incorporated anchor-piece (see Ref. 29) causes a conformational change which enhances enzyme activity; binding of bile salts to the hydrophobic anchor-piece might limit access of the proteinase, inhibit cleavage and so prevent activation.

Brush-border maltase was not quite as stable in duodenal secretions as sucrase, but neither bile salts or phospholipids in isolation, led to loss of activity. As with sucrase, the enzyme was activated by the proteo-

lytic fraction or elastase, but not trypsin, activation being largely prevented by addition of bile salts.

Release of sucrase and maltase was more rapid when bile salts were present in the incubation than when proteolytic enzymes only were present. For neither enzyme was proteolytic release from the brush border followed by rapid inactivation. Seetharam et al [14] have demonstrated that soluble forms of these enzymes are degraded at the same rate as membrane-bound forms. Trypsin had very little effect on any enzyme studied, which agrees with earlier findings [12,13]. However, when used in combination with elastase, it augmented release from the membrane of all three disaccharidases (Fig. 5), but not the rate of inactivation (Table I). The in vitro release of human sucrase and maltase from the brush border is very similar to that seen with pig, rat and rabbit membranes [29]. As enzyme release does occur in vivo [10], and as we have shown that sucrase and to a lesser degree maltase are relatively stable in duodenal secretions, then substantial quantities of these enzymes might be present in the lumen in an active form after a meal and so be of physiological significance.

Duodenal secretions had a different effect on lactase activity compared to sucrase and maltase, although the effect on release was similar. Elastase appeared the most important element leading to inactivation, but the rate of loss of activity brought about by elastase alone, in combination with trypsin, or when applied as the proteolytic fraction of duodenal secretions, was never $> 50\%$ of that due to duodenal secretions. Inactivation was only partially reversed (approx. 50%) by addition of proteinase inhibitors. Thus, there appears to be an unidentified factor in duodenal secretions which potentiates, or is directly responsible for, inactivation. The extra inhibitory activity of duodenal secretions could not be attributed to bile salts or phospholipids, nor was it a dose-effect; the proteolytic activity of the proteolytic fraction was 85% that of untreated secretions. Lactase has been shown to be more sensitive to proteolytic enzymes in vivo than in vitro [15], which is consistent with an additional potentiating factor in duodenal secretions. These results indicate that degradative luminal factors might be as important in the rate limiting hydrolysis of lactase in lactase deficiency, as is the synthesis or functional efficacy of the enzyme.

The pattern of inactivation and release of alkaline phosphatase differed from the other enzymes. Loss of activity seen in duodenal secretions was largely reversed by addition of proteolytic inhibitors and the majority of this degradative effect could be attributed to elastase, which had a similar effect to that reported by Maestracci [13]. However, we found alkaline phosphatase to be inherently unstable in the incubation buffer as opposed to the findings by Maestracci [13], even when buffer was supplemented with trace Mg^{2+} and/or Zn^{2+} (personal observations). While proteolytic enzymes had a significant degradative effect on alkaline phosphatase, relatively small amounts of active enzyme were released from the brush border, which agrees with an earlier report [14]. This would be consistent with it being more deeply buried within the brush border and less accessible than the disaccharidases.

Exposure of brush borders of nonhuman species to proteinases have shown that the hydrophobic anchor-piece which fixes an enzyme to the brush border is cleaved, thus releasing the hydrophilic, hydrolytically active part of enzymes such as sucrase and aminopeptidase into the supernatant fraction [30,31,29,8,14]. It is possible that continued exposure to proteinase might subsequently lead to further 'digestion' and loss of hydrolytic activity of the solubilised enzyme; in this context, solubilisation would be an early step in the degradation process. Our results show that human maltase and lactase are affected in this way. Sucrase, which is also released, is inactivated only very slowly; it is known to undergo proteolytic modification while still attached to the brush border [32] but this does not appear to have a major effect on hydrolytic activity. An additional sequence of events is that proteolytic damage and loss of hydrolytic activity can occur while the enzyme remains attached to the brush border, or simultaneous with its release. The results of this study suggest that alkaline phosphatase is affected in this way and that initial release from the brush border is not essential as a prerequisite to proteolytic inactivation.

Luminal agents with a detergent action, i.e., bile salts, remove enzymes by disrupting the plasma membrane and incorporating the enzyme by its hydrophobic anchor-piece into a mixed micelle [29]. The effect of micellar solubilisation on activities of human brush-border enzymes, using bile salts, has been relatively little studied. Nonionic detergents are effective in solubilisation of most human enzymes in an active form [33]. However, the detergent action of bile salts is normally operative in an environment in which proteinases are also present. The major effect of duodenal secretions on the human brush border in vitro was rapid release of all enzymes in an active form. This was reproduced by bile salts alone. The fastest losses of enzyme activities occurred in duodenal secretions, but bile salts themselves were not responsible for this. Bile salt micellar solubilisation did not prevent the eventual proteinase-mediated loss of activity of lactase or alkaline phosphatase, but the activation of sucrase and maltase by the proteolytic fraction or by elastase was, however, prevented by addition of bile salts. Thus bile salts appear to partly restrict the action of the proteinases on these enzymes.

The physiological and pathophysiological implications of these findings are dependent on the enzyme. It has long been known that brush-border enzymes play a

crucial final role in digestion at the brush border (see Ref. 9). Yet there is no reason why human maltase and sucrase, which we have shown to be relatively stable as soluble enzymes in duodenal secretions, should not perform a useful digestive function in the gut lumen. It has been shown in the rat that stimulation of pancreatic secretion by cholecystokinin and secretin releases significant amounts of sucrase and alkaline phosphatase into the lumen [10], and that sucrase and maltase are present in the lumen in soluble forms in unstimulated circumstances [34], thus components of pancreaticobiliary secretions do gain access to the brush border in vivo. While these in vitro studies expose the brush border to luminal components in an artificial manner, i.e., with overlying mucus gel largely removed, the relative susceptibilities of the brush-border enzymes to luminal components, and the interactive effect of the luminal components on brush-border enzymes, should have in vivo relevance. It should be recognized, however, that the speed and magnitude of enzyme release and/or inactivation may be different in vivo. Access of dietary substrate to solubilised enzyme in the lumen would not be restricted by the potential retarding effect of the unstirred layer overlying the cell surface [35]. It can be envisaged that maltase and sucrase at least, are 'stored' at the brush border, waiting to be partly released at meal-time by pancreaticobiliary secretions with the result that they are active both in the lumen and at the brush border.

It is already established that patients [36] as well as animals [8] with pancreatic insufficiency have increased activities of brush-border enzymes, which suggests that pancreatic enzymes are important in enzyme turnover in vivo. It has also been shown that interaction of pancreatic proteinases with lactase in lactase deficiency is significant, in that the condition is worsened [16]. Conceivably, there are lactase variants which have increased susceptibility to adverse effects by luminal factors which would be important in the rate limiting effect of lactose hydrolysis in lactase deficiency. The molecular defects in the various types of lactase deficiency have not been fully defined and the problem may not lie just with the hydrolytic function of the enzyme, but also with its susceptibility to luminal factors.

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