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Evidence of degradation process of sucrase–isomaltase in jejunum of adult rats

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To evaluate degradation processes of sucrase–isomaltase in adult rat jejunum, we determined enzymic activity of sucrase and isomaltase and compared it with the amount of immunoreactive sucrase–isomaltase. In rats fed or starved for 18h, killed at 10:00h or 22:00h, sucrase activity (expressed on the basis of total protein or of immunoreactive sucrase–isomaltase) was significantly ($P<0.02$) lower in the lower jejunum than in the upper jejunum; isomaltase activity was similar in both segments. Crossed immunoelectrophoresis demonstrated the existence of a second sucrase–isomaltase antigen reacting with anti-(sucrase–isomaltase) serum. This antigen was present in larger amounts in the lower jejunum than in the upper jejunum, exhibited immunological partial identity with the intact sucrase–isomaltase, and had isomaltase activity but no sucrase activity. Results suggest that this antigen is a degradation product of sucrase–isomaltase in which the sucrase active site has been broken down. To examine the role of pancreatic enzymes in degradation of sucrase–isomaltase, common pancreatico-biliary ducts were ligated. Within 18h after the operation, the difference of sucrase activity between the upper and the lower jejunum disappeared and the amount of the second sucrase–isomaltase antigen markedly decreased in the lower jejunum. Our results indicate that, during the degradation of intestinal sucrase–isomaltase by the pancreatic proteinases, degradation of the sucrase active site precedes that of the isomaltase active site.

The dimeric enzyme sucrase–isomaltase (a complex of sucrose α-glucohydrolase, EC 3.2.1.48, and isomaltase, EC 3.2.1.10) is synthesized as a single chain precursor protein with enzymic activity (Hauri *et al.*, 1979, 1982; Sjöström *et al.*, 1980; Montgomery *et al.*, 1981). In the intestinal brush border membrane, this precursor protein is cleaved into its mature subunits by the action of pancreatic proteinases (Hauri *et al.*, 1979; Sjöström *et al.*, 1980). Sucrase–isomaltase is bound to the brush border membrane via a hydrophobic polypeptide segment which is located near the *N*-terminal region of the isomaltase (Brunner *et al.*, 1979; Hauri *et al.*, 1982).

The activity of sucrase in jejunoileum is modified by pancreatic enzymes; sucrase activity was found to be increased in mice with pancreatic insufficiency (Kwong *et al.*, 1978) and in patients with chronic pancreatitis (Arvanitakis & Olsen, 1974). Sucrase activity also increased in pancreatectomized rats (Alpers & Tedesco, 1975) and in the rats with ligated pancreatic ducts (Riby & Kretchmer, 1984). However, in these early studies, neither immunoreactive amount of sucrase–isomaltase nor isomaltase activity was determined. Therefore, it is uncertain whether degradation of sucrase–isomaltase occurs on or off the membrane, and no information is available about the degradation of the isomaltase subunit.

In experiments recently performed in our laboratory, we observed that sucrase activity (on the basis of immunoreactive sucrase–isomaltase) was less in the lower part of jejunum than in the upper part of jejunum, whereas isomaltase activity expressed on the same basis was similar between these two segments. Assuming that the immunoreactivity of an enzyme to the corresponding antiserum is preserved more than its catalytic activity, these results implied the differential degradation of sucrase and isomaltase subunits. This present report summarizes the data from these experiments. To evaluate the role of pancreatic secretion in the degradative process of

Abbreviation used: S/I, sucrase/isomaltase activity ratio.

sucrase–isomaltase, studies were also performed on rats where the common pancreatico-biliary duct was ligated. Preliminary reports on these studies appeared elsewhere (Goda & Koldovský, 1985).

Materials and methods

Animals

Rats of the Sprague–Dawley strain (2 months old) were used. They were fed *ad libitum* a laboratory chow diet (Lab Blox, Allied Mills, Chicago, IL, U.S.A.) until the experiments were initiated. Animals, in a fed state, were killed by decapitation at either 10:00h or 22:00h. Some animals were starved for 18h and killed at 10:00h. Jejunoileum, from the ligament of Treitz to the jejuno-caecal valve, was divided into three equal parts. The distal third was discarded. The proximal third (upper jejunum) and the middle third (lower jejunum) of jejunoileum was flushed separately with 10ml of ice-cold 0.9% NaCl solution. The flush was saved and stored at −20°C for the determination of trypsin and α-amylase activity. Each intestinal segment was cut open, rinsed extensively with 0.9% NaCl solution and blotted with paper. Mucosa was scraped with a glass slide and stored at −20°C; homogenate was prepared within 3 days.

In other experiments, 18h prior to the killing, i.e. at 16:00h, the animals were anaesthetized with diethyl ether and the pancreatico-biliary ducts were ligated at the lower end of the ducts. The ligation was performed at two points of the ducts (approx. 1cm apart), and the ducts were disconnected between the ligatures (Lambert, 1965). The control animals were sham-operated. Following the operation, animals had free access to chow diet and were killed at 10:00h.

Preparation of intestinal samples

Intestinal mucosa was homogenized with 4vol. of 10mM-potassium phosphate buffer (pH7.0). Sucrase–isomaltase was solubilized from the mucosal homogenate with papain as previously described (Goda *et al.*, 1984). The papain-treated homogenate was dialysed for 16h against 10mM-potassium phosphate buffer (pH7.0) and centrifuged at 105000*g* for 60min at 4°C. This supernatant was used for the determination of sucrase and isomaltase activity and the quantification of immunoreactive sucrase–isomaltase.

Preparation of antiserum

Sucrase–isomaltase was purified from the jejunoileum of 3-month-old Sprague–Dawley rats by using the method of Kolinská & Kraml (1972). Purification of sucrase–isomaltase consisted of a sequence of steps: solubilization by papain, precipitation with $(NH_4)_2SO_4$ (45–65% saturation) and Sephadex G-200, DEAE-Sephadex A-25 and Ultrogel AcA 34 column chromatographies. This resulted in a 422-fold purification (in terms of sucrase activity) to a specific sucrase activity of 950μmol/h per mg of protein. Isomaltase activity, determined as palatinose-hydrolysing activity, of this purified enzyme was 124μmol/h per mg of protein. Homogeneity of the purified enzyme was monitored by polyacrylamide-disc-gel electrophoresis with 6% polyacrylamide (Davis, 1964); a single band was detected.

Antiserum was prepared by injecting a rabbit subcutaneously on the back with 200μg of purified sucrase–isomaltase protein mixed with 0.5ml of Freund's complete adjuvant. The injection was repeated after 2 weeks with 200μg of purified enzyme mixed with 0.5ml of Freund's incomplete adjuvant, and 5 weeks later the rabbit was bled by cardiac puncture. Monospecificity of antiserum against sucrase–isomaltase was examined by crossed immunoelectrophoresis against a papain-solubilized supernatant of small intestinal homogenate containing sucrase activity of 0.45μmol/h, maltase activity of 2.5μmol/h, lactase activity of 0.18μmol/h, and 54μg of protein in 12μl applied. This loading of antigen produced two partially fused precipitate peaks that were stained with Coomassie Brilliant Blue (see Fig. 1). The main, densely stained, precipitate peak reacted positively with enzyme staining reagents (Goda *et al.*, 1984) containing 100mM-sucrose and with the reagent containing 100mM-palatinose. The second (faint) precipitate peak (its antigen had faster mobility toward the anode on electrophoresis than had the main peak) reacted positively with the enzyme staining reagent containing 100mM-palatinose but not with that containing sucrose.

Enzyme and immunological assays

Sucrase, isomaltase, maltase and lactase activities were assayed as described by Dahlqvist (1964) with 28mM of sucrose, palatinose, maltose and lactose as substrate, respectively. Palatinose was used as a substitute of isomaltose because palatinose is known to be hydrolysed by the isomaltase subunit of sucrase–isomaltase (Dahlqvist *et al.*, 1963; Goda & Hosoya, 1983). Trypsin activity was assayed by the method of Erlanger *et al.* (1961) with 94mM-α-*N*-benzoyl-DL-arginine-*p*-nitro-anilide (Sigma) as substrate, and in the presence of 53μg of enterokinase/ml (Sigma); α-amylase activity was assayed with Phadebas amylase test tablets (Pharmacia Diagnostics) according to the manufacturer's instructions. Protein was determined by the method of Lowry *et al.* (1951). Immunoreactive sucrase–isomaltase was quantified by electroimmunoassay (rocket technique;

Laurell, 1972) with purified sucrase–isomaltase as standard and as modified recently for determination of immunoreactive lactase (Goda *et al.*, 1984). The plate was stained with Coomassie Brilliant Blue. The heights of main peaks, which could also be stained with enzyme staining reagent containing sucrose, were measured. Concentration of immunoreactive sucrase–isomaltase were extrapolated from the standard curve which was linear between 30 and 100 μg/ml.

Analytical immunoelectrophoresis

Two-dimensional immunoelectrophoresis (crossed immunoelectrophoresis) was performed on 1% agarose gel in 30mM-Tris/barbital buffer, pH8.8. Papain-solubilized supernatant (12 μl) was applied. The first dimension electrophoresis was run for 105min at 18V/cm. The second dimension electrophoresis was run against 1% agarose gel containing diluted antiserum (1:50) for 20h at 6V/cm. After electrophoresis, the glass plate was washed with 0.9% NaCl and pressed with filter paper for 15min. The glass plate was then dried and stained with Coomassie Brilliant Blue.

Statistical analysis

Statistical analysis was performed with one-way analysis of variance followed by Student's *t*-test; where appropriate, a paired *t*-test was applied.

Results

Sucrase and isomaltase activities and the immunoreactive sucrase–isomaltase in upper and lower jejunum

In fed rats killed at 10:00h, the sucrase activity in the lower jejunum was significantly ($P<0.01$) lower than in the upper jejunum. However, isomaltase activity did not significantly differ between the segments (Table 1). Consequently, the sucrase/isomaltase activity ratio (S/I) in the lower jejunum was significantly ($P<0.02$) lower than in the upper jejunum. Starvation for 18h before death did not lead to a significant change in either sucrase or isomaltase activity in upper and lower jejunum (Table 1). When rats were killed at 22:00h, i.e. in a period when rats consume considerably more food than during the morning hours, sucrase activity in the upper jejunum was higher (40%) than in fed rats killed at 10:00h. Sucrase activity in the lower jejunum in rats killed at 22:00h was again significantly lower ($P<0.01$) than in the upper jejunum. Isomaltase activity was again similar in both segments. The difference in S/I ratio between the upper and the lower jejunum was remarkable (Table 1).

In order to explore the mechanism influencing the difference in S/I ratio between the upper and the lower jejunum, sucrase–isomaltase was solubilized with papain from mucosal homogenate and the amount of immunoreactive sucrase–isomaltase

Table 1. *Activities of sucrase and isomaltase and the amount of immunoreactive sucrase–isomaltase in mucosal homogenate and in papain-solubilized supernatant in upper and lower jejunum of rats*

Results are shown as means ± S.E.M.; $n = 4$/group. Significant difference from the value obtained in upper jejunum is shown by $*P<0.05$, $**P<0.02$ and $***P<0.01$. Significant difference from the value obtained in the corresponding part of intestine of fed animals killed at 10:00h is shown by $\dagger P<0.05$. Activities of sucrase and isomaltase are given in μmol/h per mg of protein.

Feeding state ...	Fed		18h starved		Fed	
Time of killing ...	10:00h		10:00h		22:00h	
	Upper jejunum	Lower jejunum	Upper jejunum	Lower jejunum	Upper jejunum	Lower jejunum
Homogenate						
Sucrase activity	2.93±0.18	1.76±0.10***	3.11±0.27	1.69±0.19***	4.10±0.18†	1.53±0.34***
Isomaltase activity	0.90±0.08	0.75±0.06	0.84±0.04	0.82±0.03	1.04±0.06	0.89±0.06
S/I	3.31±0.24	2.37±0.09**	3.69±0.16	2.05±0.18***	3.98±0.28	1.68±0.25***†
Papain supernatant						
Sucrase activity	9.2±1.2	5.1±0.5**	10.4±0.8	5.0±0.4***	13.3±0.6†	4.8±1.4***
Isomaltase activity	2.11±0.16	1.76±0.20	2.06±0.09	1.85±0.14	2.52±0.24	2.17±0.26
Immunoreactive sucrase–isomaltase (IRS) (μg/mg of protein)	13.1±1.5	11.5±0.6	14.2±2.0	10.2±1.7	13.2±1.6	12.0±1.8
Sucrase activity/IRS (μmol/h per μg)	0.71±0.06	0.45±0.05**	0.75±0.06	0.51±0.06*	1.04±0.15	0.41±0.10***
Isomaltase activity/IRS (μmol/h per μg)	0.17±0.02	0.15±0.02	0.15±0.02	0.20±0.04	0.20±0.03	0.19±0.02

Table 2. *Trypsin activity in intestinal lumen in upper and lower jejunum of rats*
The values are expressed as μmol of *p*-nitroanilide produced/min per segment. Mean ± S.E.M. is shown; $n = 4$/group. Significant difference from the corresponding upper jejunum is shown by $^{**}P < 0.001$ by paired *t*-test.

Feeding state ...	Fed	18h starved	Fed
Time of killing ...	10:00h	10:00h	22:00h
Activity			
Upper jejunum	0.55 ± 0.05	0.36 ± 0.12	0.36 ± 0.15
Lower jejunum	1.12 ± 0.44	0.65 ± 0.18	1.26 ± 0.17**

was determined (Table 1). The enzyme activity recovered after papain treatment and centrifugation (papain-treated homogenate) was 71–82% in sucrase activity, and 61–68% in isomaltase activity, expressed as a percentage of that in the initial homogenate. This recovery was similar between the upper and the lower jejunum in any experimental group. The recovery of enzyme activities from the papain-treated homogenate (= 100%) in the 105000*g* supernatant fraction was 90–94% of sucrase activity and 80–84% of isomaltase activity. The difference between the upper and the lower jejunum of sucrase activity in papain supernatant was similar to that of intestinal homogenate (Table 1). The amount of immunoreactive sucrase–isomaltase in the lower jejunum was similar to that in the upper jejunum (Table 1). When sucrase and isomaltase activities were expressed as μmol/h per μg of immunoreactive sucrase–isomaltase, sucrase activity in the upper jejunum was significantly ($P < 0.05$) higher than that in the lower jejunum, whereas isomaltase activity was similar in both segments (Table 1).

In order to examine whether the decrease of sucrase activity in the lower jejunum is related to luminal proteinases, trypsin activity was determined in the luminal flush (Table 2). Trypsin activity in the lumen of the lower jejunum tended to be higher (1.8–2.0-fold) than that of the upper jejunum at 10:00h. At 22:00h, this difference was expressed even more; the trypsin activity in the lower jejunum was significantly ($P < 0.001$, 3.5-fold) higher than that of the upper jejunum (Table 2).

Effects of pancreatico-biliary duct ligation

In order to explore further the role of pancreatic proteinases on the degradation of sucrase–isomaltase, pancreatico-biliary ducts were ligated at 16:00h. The enzyme activities of sucrase and isomaltase, and the amount of immunoreactive sucrase–isomaltase, were determined 18h after the operation (Table 3). Although the rats had free access to food during this period, the pancreatic duct ligated animals consumed no food. Pancreatico-biliary duct ligation led to a remarkable decrease of luminal trypsin and α-amylase activity in the lower jejunum (78–85%) as well as in the upper jejunum (63–66%) when compared with sham-operated animals, indicating success of the operation (Table 3). Sucrase activity was slightly (not significantly) increased in the ligated animals compared with that in sham-operated animals. Isomaltase activity was unaffected by the ligation of pancreatic duct. The S/I activity ratio in the lower jejunum was significantly ($P < 0.05$) increased in the pancreatic duct ligated animals compared with that of control animals. In the pancreatic duct ligated animals, no significant difference of sucrase activity and S/I activity ratio between the upper and lower jejunum was observed (Table 3).

The amount of immunoreactive sucrase–isomaltase of pancreatic duct ligated animals was determined in papain-solubilized supernatant (Table 3). In this experiment, the recovery of enzyme activity in papain-treated homogenate was 72–79% (sucrase) and 70–77% (isomaltase) of that in initial homogenate. These values were unaffected by the ligation of pancreatic ducts. In two out of five of the ligated animals, however, the sucrase and isomaltase activities recovered in papain-solubilized supernatant were unusually low, e.g. 33–51% of that in papain-treated homogenate in the case of lower jejunum. When these values were excluded from calculation, the enzyme activity recovered in papain-solubilized supernatant was 94–96% (sucrase), and 90–92% (isomaltase) of that in papain-treated homogenate with no difference between sham and ligated animals. The amount of immunoreactive sucrase–isomaltase was not changed significantly by the ligation of pancreatic duct in both segments. Although sucrase activity expressed per μg of immunoreactive sucrase–isomaltase tended to increase in the ligated animals, isomaltase activity expressed per μg of immunoreactive sucrase–isomaltase was unaffected (Table 3).

A second antigen of sucrase–isomaltase reacting with anti-(sucrase–isomaltase) serum

A preliminary study with the technique of crossed immunoelectrophoresis showed that a

Table 3. *Effects of pancreatico-biliary duct ligation on sucrase and isomaltase activity in mucosal homogenate and the amount of immunoreactive sucrase–isomaltase of upper and lower jejunum of rats*

The rats were killed 18h after the operation. $n = 3$ (sham-operated) or 5 (ligated) except for the values in papain-solubilized supernatant ($n = 3$). Significant difference from the value obtained in sham-operated animals is shown by $^{*}P < 0.05$ and $^{**}P < 0.01$; significant difference from the corresponding upper jejunum is shown by $\dagger P < 0.05$ by paired t-test. Activities of sucrase and isomaltase are expressed as μmol/h per mg of protein.

	Sham-operated		Ligated	
	Upper jejunum	Lower jejunum	Upper jejunum	Lower jejunum
Trypsin in lumen (μmol/min)	0.54±0.12	1.77±0.29†	0.20±0.04*	0.39±0.06**†
α-Amylase in lumen (μmol of glucosidic linkages/min)	29.7±13.4	75.0±14.0†	10.6±5.6	11.6±3.1**
Homogenate				
Sucrase activity	2.80±0.17	2.02±0.31	3.50±0.37	3.27±0.48
Isomaltase activity	0.62±0.05	0.71±0.03	0.72±0.07	0.73±0.07
S/I	4.58±0.21	2.82±0.36†	4.83±0.17	4.43±0.44*
Papain supernatant				
Immunoreactive sucrase–isomaltase (IRS) (μg/mg of protein)	18.5±2.3	14.6±2.6	13.6±0.8	14.9±2.7
Sucrase activity/IRS (μmol/h per μg)	0.72±0.04	0.64±0.01	0.86±0.05	0.80±0.06
Isomaltase activity/IRS (μmol/h per μg)	0.15±0.004	0.20±0.01	0.17±0.01	0.18±0.01

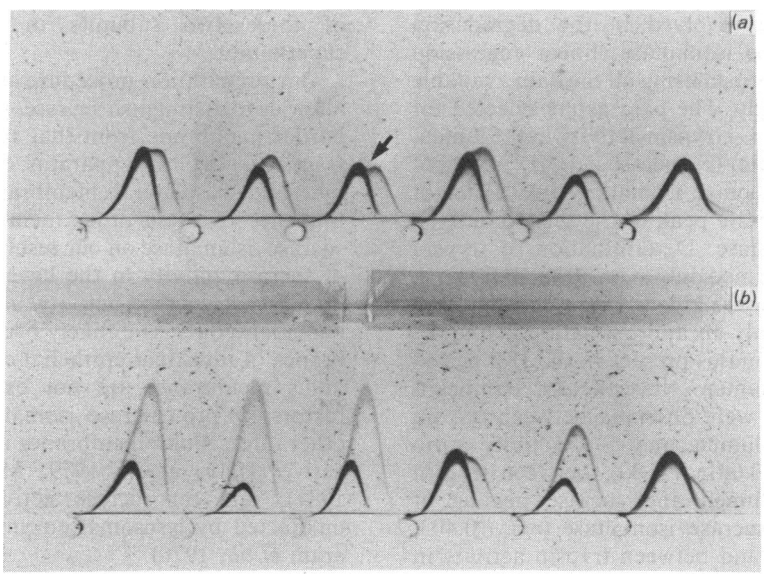

Fig. 1. *Effect of pancreatico-biliary duct ligation on the amount of the second antigen of sucrase–isomaltase in (a) the upper jejunum and (b) the lower jejunum of rats*

In the left, three wells, samples from control animals were applied. In the right three wells, samples from ligated animals were applied. After electrophoresis for 105min (anode, right) immunoelectrophoresis was performed against rabbit anti-(sucrase–isomaltase) serum. ↙ points to partial fusion of the two immunoprecipitate peaks.

second antigen reacting with anti-(sucrase–isomaltase) serum existed in intestinal homogenate. This second antigen was considered to be related with the sucrase–isomaltase because the immunoprecipitate peak of the second antigen partially fused with the main peak of sucrase–isomaltase (Fig. 1), and also monoclonal antibody to rat sucrase–isomaltase (BBC 1-35/11/2; provided by

Dr. A. Quaroni, Cornell University) bound to the second immunoprecipitate peak (results not shown).

The height of the precipitate peak of the second antigen on crossed immunoelectrophoresis was measured to quantify the relative amount of this second antigen. In control animals, the height of the second precipitate peak was markedly higher in the lower jejunum (22.9 ± 0.3 mm; mean ± S.E.M. for three animals) than that in the upper jejunum (10.6 ± 0.8 mm) (Fig. 1). In starved, as well as in fed, animals killed at 10:00h or 22:00h, the amount of the second antigen was again higher in the lower jejunum than in the upper jejunum (results not shown). The ligation of the pancreatic duct led to a marked decrease of the height of this second peak in the lower jejunum (10.5 ± 2.6 mm; three animals) (Fig. 1*b*). This value was similar to that in the upper jejunum of sham-operated animals. In the upper jejunum, the height of the second peak in the pancreatic duct ligated animals was similar to that of sham-operated animals (Fig. 1*a*).

Linear regression analysis of interparameter relations

To analyse the relation between the factors considered to be involved in the degradation process of sucrase–isomaltase, linear regression analysis was performed using all the data available in the present study. The parameters selected for the analysis were: (*a*) trypsin activity in the lumen, (*b*) S/I activity ratio, (*c*) sucrase activity per μg of immunoreactive sucrase–isomaltase and (*d*) height of immunoprecipitate peak of the second antigen of sucrase–isomaltase. Determination of trypsin activity was performed only at one time period, i.e. after the animals were killed. This single determination gives us only an approximation of the activity of the proteinases present in the 18 h period in the intestinal lumen. Nevertheless, significant linear regressions were observed between trypsin activity in the lumen and S/I activity ratio ($r = -0.563$, $P < 0.001$, $n = 39$), between trypsin activity in the lumen and sucrase per μg of immunoreactive sucrase–isomaltase ($r = -0.403$, $P < 0.05$, $n = 35$), and between trypsin activity in the lumen and the height of the second immunoprecipitate peak ($r = 0.535$, $P < 0.001$, $n = 35$). In addition, a linear regression was established between S/I activity ratio and the height of immunoprecipitate peak of the second antigen of sucrase–isomaltase ($r = -0.90$, $P < 0.001$, $n = 34$).

Discussion

Our results strongly suggest that the sucrase active site of sucrase–isomaltase is degraded by the action of pancreatic enzymes, whereas the isomaltase active site is resistant to proteolysis. For purpose of quantitative analysis of activity and immunoreactivity of sucrase–isomaltase, the yield of these activities in the preparation was crucial. Therefore, we avoided further purification of brush border fractions. Instead, sucrase–isomaltase was solubilized from mucosal homogenate. We assumed that this preparation of soluble sucrase–isomaltase was derived essentially from membrane-bound enzymes, because when fresh mucosal homogenate was immediately centrifuged at 105000*g* for 60 min at 4°C, sucrase activity recovered in the supernatant fraction was only 0–4% of that of homogenate. We used papain for the solubilization of sucrase–isomaltase from the membrane because the purified sucrase–isomaltase used as standard for electroimmunoassay was the papain-solubilized form, and the rabbit antiserum was raised against the papain-solubilized form of sucrase–isomaltase. During this solubilization procedure with papain, 18–29% of sucrase activity and 32–39% of isomaltase activity was lost. The extent of decrease in activity was similar between the upper jejunum and the lower jejunum, and was also similar between sucrase and isomaltase. Therefore, we compared the enzyme activity of these two subunits in papain-solubilized supernatant.

Our preparation procedure apparently does not allow us to distinguish sucrase–isomaltase in brush border membrane from that in other membrane fractions, i.e. Golgi apparatus, endoplasmic reticulum and basolateral membrane. However, the influence of these other membrane fractions of sucrase–isomaltase on our results, i.e. the decrease of sucrase activity in the lower jejunum and the increase of sucrase activity in rats with ligated pancreatic duct, is unlikely because (*a*) the membranes in intestinal epithelial cells, except microvillus membrane, are not exposed to luminal factors, (*b*) pro-(sucrase–isomaltase) in Golgi and other intracellular membranes is fully catalytically active (Hauri *et al.*, 1979; Montgomery *et al.*, 1981); and (*c*) sucrase activity is practically unaffected by lysosomal enzymes *in vitro* (Seetharam *et al.*, 1976).

The proposed degradation process, whereby sucrase is broken down by pancreatic enzymes, is consistent with the earlier findings of Alpers and his coworkers who observed that pancreatic insufficiency caused elevation of sucrase activity in experimental animals (Alpers & Tedesco, 1975; Kwong *et al.*, 1978). They also showed that oral administration of pancreatic enzymes in patients caused a significant decrease of sucrase activity accompanied by the decrease in maltase and lactase activities (Seetharam *et al.*, 1980).

The differential degradation of sucrase and

isomaltase subunits might be explainable by the positioning of sucrase–isomaltase to the membranes. The sucrase subunit is held in place solely by its association with the isomaltase subunit on the luminal side of the microvillus membrane (Brunner *et al.*, 1979; Hauri *et al.*, 1982). The peripheral positioning of sucrase might result in higher susceptibility of sucrase than isomaltase to luminal proteinases. However, it should be noted that once the sucrase–isomaltase is released from the membrane into the lumen in intact form, it may be degraded in a different manner: the isomaltase subunit of purified sucrase–isomaltase (soluble form) is more susceptible to tryptic digestion than sucrase subunit *in vitro*, as shown in rabbits (Quaroni *et al.*, 1975).

The model of degradation of sucrase–isomaltase, whereby the sucrase subunit is degraded prior to the degradation of the isomaltase subunit, is supported by the finding of a second antigen of sucrase–isomaltase which has isomaltase activity, but not sucrase activity (Fig. 1). This second antigen of sucrase–isomaltase is not considered to be an artifact due to papain solubilization procedure; the reasons are as follows. First, solubilization with 1% Triton X-100 and 1 mg of trypsin inhibitor/ml (Sigma type II-S) in 10 mM-potassium phosphate buffer (pH 7.0) at 4°C for 90 min produced a similar amount of this second antigen as judged from the height of the immunoprecipitate peak on crossed immunoelectrophoresis against rabbit anti-(sucrase–isomaltase) serum (T. Goda & O. Koldovský, unpublished work). Secondly, papain treatment at 37°C of mucosal homogenate for various periods of time (15–130 min) did not affect the amount of the second antigen (T. Goda & O. Koldovský, unpublished work). Thirdly, and most persuasively, the height of the immunoprecipitate peak of this second antigen to anti-(sucrase–isomaltase) serum correlated with the level of luminal trypsin. Additionally, experiments described below indicate that this second antigen originates from brush border membranes. Sucrase–isomaltase was solubilized from purified brush border membranes (prepared according to the method of Kessler *et al.*, 1978), and identical sucrase activity was applied to crossed immunoelectrophoresis; similar heights of main and second immunoprecipitate peaks were observed when they were compared with the antigens obtained from the papain-solubilized supernatant of mucosal homogenate. Therefore, we considered this second antigen of sucrase–isomaltase to be an intrinsic degradation product of sucrase–isomaltase in which the sucrase subunit has been at least partially broken down. The similar antigen of sucrase–isomaltase which lacks sucrase activity, but has isomaltase activity, has been reported in brush border membranes of human jejunum (Skovbjerg *et al.*, 1979). The second antigen of sucrase–isomaltase is distinguishable from the main (intact) antigen by its faster anodal electrophoretic mobility (Fig. 1). Partial fusion of the immunoprecipitate lines of these two antigens on crossed immunoelectrophoresis (Fig. 1) suggests that the second antigen lacks at least one of its antigenic determinants.

The difference of S/I activity ratio between the upper jejunum and the lower jejunum (Tables 1 and 3) is striking. This difference seems to be caused by the differential level of luminal proteolytic enzymes. The evidence that the luminal trypsin activity is higher in the lower jejunum than in the upper jejunum (Tables 2 and 3) favours this explanation. The reason why the activity of pancreatic enzyme is higher in the lower jejunum than in the upper jejunum is not clear. Difference in propulsion of chyme between the upper and the lower jejunum can be considered. In animals fed lab chow diet, the lumen of the lower part of jejunoileum is usually full of chyme whereas that of upper jejunum is almost empty. Pancreatic proteinases are considered to be mixed with the chyme and transferred with it. Therefore, the luminal surface of the upper jejunal mucosa might be exposed to pancreatic enzymes for a relatively shorter period than that of the lower jejunum.

The difference between the upper and the lower jejunum regarding its sucrase and isomaltase activity might be physiologically significant. Sucrase–isomaltase plays an important role for digestion of not only sucrose, but also α-limit dextrins (digestion products of starch) containing both α-(1-4) linkages and one or more α-(1-6) branching links (Gray *et al.*, 1979; Rodriguez *et al.*, 1984). No preprocessing of sucrose is necessary prior to final digestion by sucrase. Sucrose digestion may occur in duodenum and in the very upper part of jejunum where sucrase activity is preserved as shown in the present study. Digestion of starch is a more complicated and time-consuming process because of its large size. As a part of the final digestive process of starch, α-(1-6) branching links of α-limit dextrins are split by isomaltase. It is advantageous, therefore, that isomaltase activity is fully preserved in the lower part of jejunum by its resistance to proteolysis.

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