Characterization of degradation process of sucrase-isomaltase in rat jejunum with monoclonal-antibody-based enzyme-linked immunosorbent assay

Toshinao GODA,* Andrea QUARONI† and Otakar KOLDOVSKÝ*‡

*Neonatology and Nutritional Sciences Section, Department of Pediatrics and Physiology, University of Arizona College of Medicine, Tucson, AZ 85724, and †Section of Physiology, Division of Biological Sciences, Cornell University, Ithaca, NY 14853, U.S.A.

As shown previously, during degradation of sucrase-isomaltase in rat jejunum, degradation of the sucrase active site occurs before that of isomaltase active site [Goda & Koldovský (1985) *Biochem. J.* **229**, 751–758]. To characterize further the process of sucrase-isomaltase degradation in jejunum, we determined the amounts of immunoreactive sucrase-isomaltase in rat jejunum by using a monoclonal-antibody-based enzyme-linked immunosorbent assay. By employing two alternative monoclonal antibodies (one reacting with the sucrase subunit and the other reacting with the isomaltase subunit), the amount of antigen-containing sucrase subunit and the amount of antigen-containing isomaltase subunit were separately quantified. In both upper and lower jejunum of rats, the amount of antigen-containing isomaltase subunit was always higher than the amount of antigen-containing sucrase subunit. This difference was attributable mainly to a degradation product of sucrase-isomaltase, which was identified as isomaltase monomer. Occlusion of pancreatic ducts for 18 h eliminated the difference between the amount of antigen-containing sucrase subunit and the amount of antigen-containing isomaltase subunit in both upper and lower jejunum. In jejunum of control animals, the molar ratio of sucrase subunit to isomaltase subunit was estimated to be 0.32–0.52, indicating that quite a large proportion of sucrase-isomaltase (48–68 %) is present as degradation products (e.g. isomaltase monomer). These results support the model of degradation process of sucrase-isomaltase in brush-border membranes of rat jejunum, whereby degradation of sucrase subunit by the action of pancreatic proteinase(s) precedes degradation of isomaltase subunit.

INTRODUCTION

The dimeric enzyme sucrase-isomaltase is synthesized as a single-chain precursor protein in small-intestinal epithelial cells (Hauri *et al.*, 1979, 1982; Sjöström *et al.*, 1980; Montgomery *et al.*, 1981; Hunziker *et al.*, 1986). 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 moiety (Brunner *et al.*, 1979; Semenza, 1986). Previously we demonstrated the existence of a second sucrase-isomaltase antigen reacting with anti-(sucrase-isomaltase) serum. Since the amount of this antigen was remarkably diminished in rats with ligated pancreatic ducts, we considered this second antigen to be a degradation product of sucrase-isomaltase (Goda & Koldovský, 1985). In the present paper we characterize this degradation product of sucrase-isomaltase which is present in brush-border membrane of rat jejunum. To characterize further the degradation process of sucrase-isomaltase, we developed a monoclonal antibody-based enzyme-linked immunosorbent assay (e.l.i.s.a.). The amount of immunoreactive sucrase was compared with that of immunoreactive isomaltase in control rats as well as in rats with occluded pancreatic ducts.

MATERIALS AND METHODS

Animals

Male rats of the Sprague–Dawley strain (7 months old) were fed a standard laboratory chow diet (Lab Blox, Allied Mills, Chicago, IL, U.S.A.) *ad libitum* until the experiments were initiated. Animals were anaesthetized 18 h before they were killed with diethyl ether; the pancreato-biliary duct was ligated at the lowest end and, subsequent to this, the upper bile duct was bypassed to the duodenum with a polyethylene catheter (Lambert, 1965) (control animals were sham-operated). After the operation, animals were starved and killed at 1000 h. The jejunoileum (from the ligament of Treitz to the ileo-caecal valve) was divided into three equal segments. The proximal third (upper jejunum) and middle third (lower jejunum) was flushed separately with ice-cold 0.9 % NaCl solution. The intestinal segment was cut with scissors along its length, and blotted with wet soft tissue paper. Mucosa was scraped with a glass slide and stored at -20°C for further analysis.

Preparation of intestinal samples

Intestinal mucosa was homogenized with 4 vol. of 10 mM-potassium phosphate buffer, pH 7.0. Sucrase-isomaltase was solubilized from the mucosal homogenate with papain as described previously (Goda *et al.*, 1984).

Abbreviation used: e.l.i.s.a., enzyme-linked immunosorbent assay.

‡ To whom correspondence and reprint requests should be addressed.

The papain-treated homogenate was dialysed for 16 h against 10 mM-potassium phosphate buffer, pH 7.0, and centrifuged at 105000 *g* for 60 min at 4 °C. This supernatant was used for the determination of sucrase and isomaltase activity and the quantification of immunoreactive sucrase-isomaltase and isomaltase monomer.

Separation of sucrase-isomaltase and its degradation product

Sucrase-isomaltase was solubilized from the 60000 *g* particulate fraction of lower-jejunal mucosa of 4-month-old male Sprague-Dawley rats with papain as described by Kolinská & Kraml (1972). The papain-solubilized sucrase-isomaltase was applied on a column (2.5 cm × 95 cm) of Ultrogel AcA 34 (LKB, Villeneuve-Garenne, France) that had been equilibrated with 50 mM-potassium phosphate buffer, pH 7.0 (flow rate 10 ml/h). Fractions (5 ml) were collected and portions were used for enzyme and immunological assays. The third peak of isomaltase activity, which lacks sucrase activity (referred to as 'isomaltase monomer'), was identified (see Fig. 2 below) and its fractions pooled and concentrated in an Amicon model 52 apparatus with a PM 10 membrane. The isomaltase monomer was further purified with a Sephadex G-200 (superfine grade) column as described by Cézard *et al.* (1979), followed by subsequent column-chromatographic separations on Ultrogel AcA 34 and DEAE-cellulose (with a linear gradient of potassium phosphate from 10 mM to 100 mM at pH 7.0). Homogeneity of the purified isomaltase monomer was monitored by 6%-(w/v)-polyacrylamide-disc-gel electrophoresis (Davis, 1964). SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970). The following *M_r* standards (Sigma, St. Louis, MO, U.S.A.) were used: myosin (205000), β -galactosidase (116000), phosphorylase *b* (97400), lactoferrin (77000) and bovine serum albumin (66000).

Enzyme assays

Sucrase, isomaltase and maltase activities were assayed as described by Dahlqvist (1964), with 28 mM-sucrose, -palatinose and -maltose as substrate respectively. Palatinose was used as a substrate of isomaltase, since it is known to be hydrolysed by the isomaltase subunit of sucrase-isomaltase (Dahlqvist *et al.*, 1963; Goda & Hosoya, 1983). Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Preparation of antiserum

Sucrase-isomaltase was purified from the jejuno-ileum of 3-month-old Sprague-Dawley rats by the method of Kolinská & Kraml (1972), as modified by Goda & Koldovský (1985). The specific antiserum against sucrase-isomaltase was raised in a rabbit immunized with purified sucrase-isomaltase as described previously (Goda & Koldovský, 1985).

Preparation of monoclonal antibodies

Monoclonal antibodies against rat sucrase-isomaltase were prepared as described previously (Quaroni, 1983). Two monoclonal antibodies specific to sucrase-isomaltase, namely BBC1-35/11/2 and CC4-11/11/6, were selected for the present study. Both monoclonal antibodies were of the IgG₁ type. The hybridoma cells producing the monoclonal antibodies were injected in

BALB/c mice administered 2,6,10,14-tetramethylpentadecane (Pristane; Sigma, St. Louis, MO, U.S.A.) 2 weeks previously; ascites fluid was used for immunological assays of sucrase-isomaltase.

Immunological assays

Immunoreactive sucrase-isomaltase was quantified either (a) by electroimmunoassay (rocket technique; Laurell, 1972) in agarose gel as described previously (Goda & Koldovský, 1985) or (b) by e.l.i.s.a. Monoclonal antibodies were employed as captive antibodies for the latter. Briefly, the sample was applied to wells of a microtitre plate (A/2; Costar, Cambridge, MA, U.S.A.) that had been precoated with monoclonal antibody. Immobilized sucrase-isomaltase was then detected by sequential incubation of anti-(sucrase-isomaltase) serum, goat anti-rabbit IgG-peroxidase conjugate (American Qualex, La Miranda, CA, U.S.A.) and 2,2-azinodi-(3-ethylbenzthiazolinesulphonic acid) diammonium salt. Purified sucrase-isomaltase was used as standard. The detection limit of sucrase-isomaltase was 2 ng/ml with BBC1-35/11/2, and 20 ng/ml with CC4-11/11/6 as captive antibody. Immunoreactive isomaltase monomer was quantified by electroimmunoassay using purified isomaltase monomer as standard. The height of the second peak of immunoprecipitate, which corresponds to isomaltase monomer, was measured (see Fig. 2 below). In animals with occluded pancreatic ducts, the height of the second peak was smaller than that of the first (sucrase-isomaltase) peak and the amount of isomaltase monomer could not be determined.

Analytical immunoelectrophoresis

Two-dimensional immunoelectrophoresis (crossed immunoelectrophoresis) was performed against anti-(sucrase-isomaltase) serum as described previously (Goda & Koldovský, 1985).

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

Characterization of the degradation product of sucrase-isomaltase

Our previous study demonstrated the presence of a second antigen of sucrase-isomaltase in brush-border membrane of rat jejunum (Goda & Koldovský, 1985). Since occlusion of pancreatic biliary ducts remarkably diminished the amount of this antigen, we speculated that this antigen was a degradation product of sucrase-isomaltase (Goda & Koldovský, 1985). To characterize this antigen of sucrase-isomaltase, brush-border proteins in the lower-jejunal mucosa of rats were solubilized with papain and separated by gel filtration on an Ultrogel AcA 34 column. As shown in Fig. 1(a), three distinct disaccharidases possessing isomaltase activity were separated. These were referred to as 'peak I', 'peak II' and 'peak III' respectively. A single peak of sucrase activity was seen corresponding to peak II, where the greatest quantity of isomaltase activity was recovered. In peak III, a remarkable amount of isomaltase activity (35% of total isomaltase activity applied) was recovered, but

Fig. 1. Gel-filtration chromatography on an Ultrogel AcA 34 column (2.5 cm $\times$ 95 cm) of papain-solubilized brush-border proteins from lower jejunum of rats

The column was eluted with 50 mM-potassium phosphate buffer, pH 7.0, at 10 ml/h. Fractions (5 ml) were analysed for (a) sucrase (●), isomaltase (○) and maltase (△) activities and (b) the immunoreactive amount of sucrase-isomaltase determined by monoclonal-antibody-based e.l.i.s.a. using CC4-11/11/6 (●) and BBC1-35/11/2 (○). Fractions containing isomaltase activity were divided into four parts: I, II, II' and III.

these fractions did not contain detectable amounts of sucrase activity. Peak I contained a large amount of maltase activity, with a slight isomaltase activity (less than 5% of isomaltase activity applied). Peaks I, II and III represent glucoamylase-maltase complex, sucrase-isomaltase complex and isomaltase monomer respectively. The glucoamylase-maltase complex is known to possess a slight isomaltase activity (Semenza, 1986). The immunoreactive amount of sucrase-isomaltase was determined by the elution of the Ultrogel AcA 34 column by a monoclonal-antibody-based e.l.i.s.a. technique (Fig. 1b). Two monoclonal antibodies prepared against rat sucrase-isomaltase (CC4-11/11/6 and BBC1-35/11/2) were used as captive antibodies. As shown in Fig. 1(b), CC4-11/11/6 reacted with sucrase-isomaltase complex, but not with isomaltase monomer. In contrast, BBC1-35/11/2 reacted with both sucrase-isomaltase complex and isomaltase monomer. Immunoreactive sucrase-isomaltase was not detected in peak I (Fig. 1b). This

Fig. 2. Crossed immunoelectrophoresis of fractions recovered from Ultrogel AcA 34 gel filtration (cf. Fig. 1)

The same amount of isomaltase activity (0.052 $\mu\text{mol/h}$) in pooled fractions (I, II, II' and III) was applied to each well. After electrophoresis for 105 min (anode, right), immunoelectrophoresis was performed against rabbit anti-(sucrase-isomaltase) serum.

result indicates that CC4-11/11/6 binds to an epitope located in the sucrase subunit and that BBC1-35/11/2 binds to an epitope located in the isomaltase subunit.

In order to identify the second antigen reacting with anti-(sucrase-isomaltase) serum demonstrated in the previous study (Goda & Koldovský, 1985), the three peaks obtained from Ultrogel AcA 34 column chromatography were concentrated separately and analysed by crossed immunoelectrophoresis (Fig. 2). Peak II (sucrase-isomaltase) produced a single immunoprecipitation peak. Peak III (isomaltase monomer) also produced a single immunoprecipitation peak, but the electrophoretic mobility of this antigen was higher than that of the antigen present in peak II. Intermediate fractions between peaks II and III (i.e. II' in Fig. 1a) undoubtedly contained two distinct antigens. Peak I did not produce an immunoprecipitation peak with anti-(sucrase-isomaltase) serum (Fig. 2). This result indicated that the second antigen reacting with anti-(sucrase-isomaltase) serum was isomaltase monomer that lacked the sucrase subunit. The isomaltase monomer obtained in peak III was further purified and partially characterized. Homogeneity of the purified isomaltase monomer was monitored by polyacrylamide-disc-gel electrophoresis; a single band was detected (results not shown). SDS/polyacrylamide-gel electrophoresis exhibited a major band with estimated molecular size of 131 kDa and a minor band with an estimated molecular size of 100 kDa. Both molecules possessed BBC1-35/11/2-binding activity, as revealed by immunoaffinity column chromatography using BBC1-35/11/2 antibody bound to a Protein A-Sepharose 4B column (results not shown). The activity of the isomaltase monomer was 212 $\mu\text{mol/h per mg}$ of protein with palatinose as substrate and 557 $\mu\text{mol/h per mg}$ of protein with maltose as substrate. The palatinose-hydrolysing activity of the isomaltase monomer was twice as high as that of purified sucrase-isomaltase ($107 \pm 9 \mu\text{mol/h per mg}$ of protein; mean $\pm$ S.E.M. for three preparations). Inasmuch as the size of the isomaltase monomer was approximately half the size

Fig. 3. Comparison of immunoreactivity of sucrase-isomaltase and isomaltase monomer in BBC1-35/11/2-based e.l.i.s.a.

A series of (protein) concentrations of purified sucrase-isomaltase (S-I, ●) and purified isomaltase (I, ○) monomer was applied to wells of microtitre plates, which were precoated with monoclonal antibody BBC1-35/11/2. Rabbit anti-(sucrase-isomaltase) serum was then applied and the bound rabbit IgG was detected by goat anti-rabbit IgG-peroxidase conjugate, followed by colour development with 2,2-azino-di-(3-ethylbenzthiazolinesulphonic acid diammonium salt). The line connecting the points is hand-drawn.

of purified sucrase-isomaltase (two subunits of 137 kDa and 120 kDa), we considered that it retained full catalytic activity.

Quantification of sucrase-isomaltase and isomaltase monomer by e.l.i.s.a.

It is possible that the immunoreactive amount of sucrase-isomaltase determined by BBC1-35/11/2-based e.l.i.s.a. is affected by the proportion of isomaltase monomer compared with that of sucrase-isomaltase, since jejunal mucosa contains not only sucrase-isomaltase but isomaltase monomer as well. To examine whether immunoreactivity of isomaltase monomer is different from that of sucrase-isomaltase, identical amounts of purified isomaltase monomer and sucrase-isomaltase protein were applied in wells of microtitre plates which were precoated with BBC1-35/11/2. As shown in Fig. 3, the isomaltase monomer produced the same level of absorbance with half the amount of protein as compared with sucrase-isomaltase. This result suggests that sucrase subunit of sucrase-isomaltase contributes little if any to development of colour in the present system, i.e. one unit of isomaltase monomer exhibited a similar immunoreactivity compared with the same unit of sucrase-isomaltase in the BBC1-35/11/2-based e.l.i.s.a.

Effect of pancreatic-duct occlusion on immunoreactive sucrase-isomaltase

To characterize the degradation process of sucrase-isomaltase in jejunum, pancreatic ducts were occluded in rats and the activity of sucrase and isomaltase, and the immunoreactive amount of sucrase-isomaltase and isomaltase monomer, were determined in papain-solubilized supernatant of jejunal mucosa. These results are summarized in Table 1. The recovery of enzyme activity in papain-solubilized supernatant was 56–71 % (sucrase) and 60–69 % (isomaltase) of that in initial homogenate. These values were similar to those reported previously (Goda & Koldovský, 1985) and were unaffected by the occlusion of pancreatic ducts. In lower jejunum, both sucrase and isomaltase specific activities (per mg of protein) were significantly ($P < 0.05$) increased (3.8-fold in sucrase; 1.9-fold in isomaltase) in the pancreatic-duct-occluded animals compared with those in sham-operated animals. A slight (but not significant) increase in sucrase and isomaltase activity was seen also in upper jejunum of pancreatic-duct-occluded animals. The sucrase/isomaltase activity ratio (S/I) was significantly increased in both upper and lower jejunum by the occlusion of pancreatic ducts (Table 1).

The amount of immunoreactive sucrase-isomaltase was determined by three different procedures. Electro-immunoassay demonstrated the presence of significantly lower amounts of immunoreactive sucrase-isomaltase in lower jejunum than that found in upper jejunum in controls. Occlusion of pancreatic ducts led to an increase of immunoreactive sucrase-isomaltase in upper (1.9-fold) and in lower jejunum (5.1-fold). In pancreatic-duct-occluded animals, the immunoreactive amount of sucrase-isomaltase in lower jejunum was similar to that in upper jejunum (Table 1). E.l.i.s.a. of sucrase-isomaltase, using CC4-11/11/6 as captive antibody, exhibited similar values to those obtained by electroimmunoassay in both upper and lower jejunum. In contrast, e.l.i.s.a. of sucrase-isomaltase using BBC1-35/11/2 as captive antibody in sham-operated animals exhibited significantly higher values in upper ($P < 0.02$) as well as in lower jejunum ($P < 0.01$) than values obtained by either electro-immunoassay or CC4-11/11/6-based e.l.i.s.a. In sham-operated animals, the amount of antigen reacting with BBC1-35/11/2 in lower jejunum was half that observed in upper jejunum. The occlusion of pancreatic ducts led to a remarkable increase in the amount of BBC1-35/11/2-reacting antigen in lower jejunum compared with that found in upper jejunum (Table 1). In pancreatic-duct-occluded animals, all immunoassay procedures employed in the present study produced similar values of immunoreactive sucrase-isomaltase in both upper and lower jejunum (Table 1). A tight correlation was observed between sucrase activity and CC4-11/11/6-based immunoreactivity ($r = 0.90$; $P < 0.001$) as well as between isomaltase activity and BBC1-35/11/2 ($r = 0.95$; $P < 0.001$).

Since the same units of sucrase-isomaltase and isomaltase monomer produce the same immunoreactivity in BBC1-35/11/2-based e.l.i.s.a. (Fig. 3), the ratio of CC4-11/11/6-reactive and BBC1-35/11/2-reactive sucrase-isomaltase represents the molar ratio of sucrase subunit (S) and isomaltase subunit (I). The estimated S/I molar ratio was significantly higher ($P < 0.01$) in upper jejunum (0.52) than in lower jejunum (0.32) in sham-operated

Table 1. Effects of pancreatico-biliary-duct occlusion on activities of sucrase and isomaltase and the amounts of immunoreactive sucrase-isomaltase in papain-solubilized supernatant of mucosal homogenate in upper and lower jejunum of rats

The rats were killed 18 h after surgery. Results are shown as means $\pm$ S.E.M.; $n = 4$ for each group. Significant difference from the value obtained in sham-operated animals is shown by: * $P < 0.05$, ** $P < 0.01$. Significant difference from the corresponding upper jejunum is shown by: † $P < 0.05$, †† $P < 0.01$ by paired t test. Abbreviation: ND, not determined.

Parameter	Sham-operated		Occluded	
	Upper jejunum	Lower jejunum	Upper jejunum	Lower jejunum
Sucrase activity ($\mu\text{mol/h}$ per mg of protein) (S)	7.46 $\pm$ 0.59	3.30 $\pm$ 0.49††	14.92 $\pm$ 3.09	12.48 $\pm$ 2.50*
Isomaltase activity ($\mu\text{mol/h}$ per mg of protein) (I)	2.33 $\pm$ 0.23	1.59 $\pm$ 0.19†	2.96 $\pm$ 0.44	3.09 $\pm$ 0.56*
S/I	3.31 $\pm$ 0.43	2.15 $\pm$ 0.42	4.91 $\pm$ 0.35*	4.05 $\pm$ 0.24**
Immunoreactive sucrase-isomaltase ($\mu\text{g/mg}$ of protein) by:				
Electroimmunoassay	11.37 $\pm$ 2.07	4.68 $\pm$ 0.59†	22.15 $\pm$ 1.95	23.73 $\pm$ 4.66**
E.l.i.s.a.				
CC4-11/11/6 (a)	10.17 $\pm$ 0.56	3.25 $\pm$ 0.62††	27.34 $\pm$ 7.77	22.70 $\pm$ 5.42*
BBC1-35/11/2 (b)	20.41 $\pm$ 1.57	11.15 $\pm$ 1.08††	26.61 $\pm$ 4.69	27.98 $\pm$ 3.50**
S/I molar ratio (a/b)	0.52 $\pm$ 0.09	0.32 $\pm$ 0.07††	1.02 $\pm$ 0.18*	0.80 $\pm$ 0.13†
Immunoreactive isomaltase monomer by electroimmunoassay ($\mu\text{g/mg}$ of protein)	6.99 $\pm$ 1.47	5.99 $\pm$ 1.01	ND	ND

animals (Table 1). Occlusion of pancreatic ducts led to a significant increase of the S/I molar ratio in both upper and lower jejunum (Table 1).

The immunoreactive amount of isomaltase monomer quantified by electroimmunoassay was similar between upper ($6.99 \pm 1.47 \mu\text{g/mg}$ of protein) and lower jejunum (5.99 ± 1.01) in sham-operated animals (Table 1). Assuming that isomaltase monomer is the predominating degradation product and that the amounts of other forms of degradation product are negligible, the sucrase/isomaltase molar ratio was estimated from the amount of immunoreactive sucrase-isomaltase and amount of immunoreactive isomaltase monomer determined by electroimmunoassay. The formula is:

$$\text{SI}/[\text{SI} + (2 \times \text{I})]$$

where SI and I are immunoreactive sucrase-isomaltase and isomaltase monomer in $\mu\text{g/mg}$ of protein respectively. The estimated sucrase/isomaltase molar ratios in sham-operated animals were 0.45 ± 0.05 in upper jejunum and 0.35 ± 0.05 in lower jejunum. These values are similar to those calculated from monoclonal-antibody-based e.l.i.s.a. (Table 1).

DISCUSSION

Monoclonal antibodies have been used successfully as captive antibodies of solid-phase 'sandwich' enzyme immunoassay (Gupta *et al.*, 1985; Usuda *et al.*, 1986).

We used two monoclonal antibodies as captive antibodies for e.l.i.s.a. of sucrase-isomaltase. When CC4-11/11/6 is used as a captive antibody, this system detected sucrase-isomaltase complex, but failed to detect isomaltase monomer. By contrast, BBC1-35/11/2-based e.l.i.s.a. detected isomaltase monomer as well as sucrase-isomaltase complex. Therefore, we concluded that these

two monoclonal antibodies bind different epitopes of sucrase-isomaltase; CC4-11/11/6 binds to an epitope located in sucrase subunit, whereas BBC1-35/11/2 binds to an epitope located in isomaltase subunit.

The following experiments suggest that these epitopes are different from the antigenic determinants to which polyclonal antibodies (in antiserum) bind. Microtitre wells were precoated with purified sucrase-isomaltase over the range of 2–40 ng/ml and then the wells were incubated with either monoclonal antibody, followed by incubation with rabbit anti-(sucrase-isomaltase) serum. The bound rabbit IgG was detected with anti-rabbit IgG-peroxidase conjugate. The preincubation of microtitre wells with monoclonal antibodies, either CC4-11/11/6 or BBC1-35/11/2, did not inhibit the subsequent colour development (T. Goda, A. Quaroni & O. Koldovský, unpublished work). Furthermore, CC4-11/11/6 and BBC1-35/11/2 did not inhibit sucrase or isomaltase activity of purified sucrase-isomaltase (T. Goda, A. Quaroni & O. Koldovský, unpublished work). Therefore the epitopes for CC4-11/11/6 and BBC1-35/11/2 are thought to be present at sites on sucrase-isomaltase which are different from both the sucrase and the isomaltase catalytic sites. Thus four distinct sites were identified on the surface of sucrase-isomaltase: sucrase catalytic site, CC4-11/11/6-reacting epitope, isomaltase catalytic site and BBC1-35/11/2-reacting epitope. The former two sites are present on sucrase subunit and the latter two sites on isomaltase subunit.

The present study demonstrated that a considerable amount of isomaltase monomer (35–68% of total isomaltase) is present in brush-border membranes of rat jejunum (Table 1 and Fig. 1). This isomaltase monomer was identical with the second antigen detected by anti-(sucrase-isomaltase) serum which was shown previously to be a degradation product of sucrase-isomaltase (Goda

& Koldovský, 1985). As discussed previously (Goda & Koldovský, 1985), the papain-solubilization procedure does not appear to affect the amount of isomaltase monomer in small-intestinal preparations. Solubilization of sucrase-isomaltase with 1% Triton X-100 in the presence of trypsin inhibitor produced a similar amount of isomaltase monomer. Further, papain treatment at 37 °C of mucosal homogenate for various periods of time did not affect the amount of the isomaltase monomer (Goda & Koldovský, 1985). In accordance with the presence of isomaltase monomer, the immunoreactive amount of sucrase-isomaltase detected for an epitope on the isomaltase subunit was always greater than that detected for an epitope on the sucrase subunit. These findings support the proposed degradation process, whereby the sucrase subunit is degraded at a greater rate than the isomaltase subunit in brush-border membranes (Goda & Koldovský, 1985). Since a strong correlation was observed between sucrase activity and CC4-11/11/6-based immunoreactivity, as well as between isomaltase activity and BBC1-35/11/2-based immunoreactivity, disappearance from brush-border membranes of sucrase catalytic site appears to occur at a similar rate to the disappearance of CC4-11/11/6-binding epitope. The parallel behaviour of catalytic activity and epitope-based immunoreactivity of sucrase might indicate that pancreatic proteinase(s) degrade a large part of the sucrase subunit in a relatively non-specific manner. An alternative possibility is that release of the sucrase subunit from isomaltase subunit might occur. In this regard it should be noted that sucrase monomer was found in the lumen of ileum of rats (Abe *et al.*, 1985). However, it is possible that this sucrase monomer might originate from sucrase-isomaltase complex found in lumen (Abe *et al.*, 1985). It is known that isomaltase is more susceptible to proteolysis than is sucrase after sucrase-isomaltase is solubilized from the membrane (Quaroni *et al.*, 1975; Abe *et al.*, 1985). It should be explored by further study whether sequential degradation of sucrase subunit occurs in the brush-border membranes.

In lower jejunum, not only the sucrase-subunit/isomaltase-subunit molar ratio but also the immunoreactive amount and enzymic activity of sucrase-isomaltase are lower than those found in upper jejunum (Table 1). Since occlusion of pancreatic ducts eliminated this difference between upper and lower jejunum, pancreatic proteinase(s) seem to play a major role in this difference. In fact the amount of luminal trypsin activity in rats was found to be higher in lower jejunum than in upper jejunum (Goda & Koldovský, 1985; Goda *et al.*, 1985a). At present it is not clear which pancreatic proteinase is responsible for degradation and/or cleavage of the sucrase subunit. It is also not clear whether the isomaltase subunit is degraded by the action of the same pancreatic proteinase(s) as those that are responsible for degradation of the sucrase subunit.

Sucrase/isomaltase activity ratio represents a reliable indicator of the extent of degradation of sucrase-isomaltase, since isomaltase subunit is more resistant to proteolysis than sucrase subunit in the brush-border membranes of rat jejunum. Therefore the sucrase/isomaltase activity ratio might be used as a convenient indicator to assess the relative extent of degradation of

this enzyme protein *in vivo*. We recently observed that the sucrase/isomaltase activity ratio is altered by feeding of a low-starch high-protein diet (T. Goda, F. Gossé, F. Raul & O. Koldovský, unpublished work) and by feeding of a low-starch high-fat diet (Goda *et al.*, 1985b). In both cases the sucrase/isomaltase activity ratio decreased, suggesting that feeding a low-starch diet with either high protein or high fat accelerates degradation of sucrase-isomaltase in the brush-border membranes of rat small intestine.

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