CHANGES OF IMMUNOREACTIVE SUCRASE-ISOMALTAZE COMPLEX IN RAT SMALL INTESTINE DURING POSTNATAL DEVELOPMENT AND MATURATION ALONG THE VILLUS-CRYPT AXIS

Kazuhiko YAMADA, Norimasa HOSOYA, Setsuko NODA,* and Sachiko MORIUCHI**,†

Department of Nutrition, School of Health Sciences, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
*Department of Anatomy, Tokyo Women's Medical College, Shinjuku-ku, Tokyo 160, Japan
**Department of Food and Nutrition, Japan Women's University, Bunkyo-ku, Tokyo 112, Japan
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Summary Appearance of immunoreactive sucrase-isomaltase complex was observed in rat small intestine during postnatal development and maturation along the villus-crypt axis by single radial immunodiffusion. The immunoreactive sucrase-isomaltase complex in brush-border membrane increased in parallel with enzyme activities until weaning. After weaning, higher amounts of the immunoreactive enzyme proteins were found as compared with their activities. On the other hand, during cell maturation in adult rat jejunum, the immunoreactive enzyme proteins increased with the activities of sucrase-isomaltase complex. However, a significant amount of the immunoreactive enzyme proteins was observed in the crypt cells with low enzyme activities. Chromatographic profiles on Sephadex G-200 column of the sucrase-isomaltase complex in the upper villus and crypt cells did not change.

From these results, it is suggested that the appearance of sucrase and isomaltase activities until weaning is ascribed to the synthesis of an active sucrase-isomaltase complex or the synthesis of an inactive proenzyme followed by rapid conversion to active enzyme, and during cell maturation, it is caused by the synthesis of the inactive proenzyme in the crypt cells followed by its activation in the villus cells.

Keywords sucrase-isomaltase complex, immunoreactive sucrase-isomaltase complex, postnatal development, cell maturation, villus, crypt, inactive proenzyme

†山田和彦，細谷憲政，野田節子，森内幸子

171
Membrane digestive enzymes are localized on the brush-border membranes of enterocytes, and are subjected to changes in their activities by various factors. The most remarkable changes are observed in the course of development (1, 2), and of maturation of epithelial cells during migration from the crypt up to the villus tip (3).

Sucrase is a membrane digestive enzyme, which is induced at the time of weaning in mammals (4, 5), and increases its activity during maturation of epithelial cells along the villus-crypt axis (6). Furthermore, it is demonstrated that sucrase forms an enzyme-enzyme complex with isomaltase on the intestinal brush-border membranes (7-9). However, it is not known how sucrase and isomaltase appear as a complex in the course of development and of epithelial cell maturation.

Dubs et al. reported that a catalytically inactive sucrase antigen was observed in the small intestinal mucosa of young rabbits still lacking sucrase activity and on the enterocytes of mucosal crypts in adult rabbits (10). This observation suggests that sucrase and isomaltase are synthesized as an inactive protein and then subjected to modification into a catalytically active protein. However, it is still controversial whether the appearance of enzyme activities is the result of modification of pre-existing proteins in the enterocytes rather than synthesis of new proteins (11).

In our previous study, we demonstrated that sucrase and isomaltase appear simultaneously as a complex with molecular weight about 200,000 daltons, at 15 days after birth, in rat intestine (12). The present study was undertaken to determine quantitatively immunoreactive sucrase-isomaltase complex during postnatal development and maturation along the villus-crypt axis of rat small intestine, and the results are discussed with changes in enzyme activities during these processes.

METHODS

1. Animals. Nonstarved adult rats weighing 200 g and suckling rats of Wistar strain were used for the experiment. The suckling rats remained with their mothers until the following developmental ages were reached: 12, 15, 18, 21 and 25 days. Water and laboratory chow (Oriental Yeast Co.) were given ad libitum. Rats were killed by decapitation between 13:00 and 15:00 hr.

2. Preparation of brush-border membrane. Small intestine of adult rats and of rats of different ages were removed. The mucosal scraping of one animal was used for preparation of brush-border membranes, but three to four scrapings were pooled for ages younger than 18 days. The brush-border membranes were prepared from the mucosal scraping according to the method of Kessler et al. (13). Mucosal scrapings were suspended in 30 vol of ice-cold 50 mM mannitol in 2 mM Tris-HCl buffer (pH 7.1) and homogenized in a Waring blender at maximum speed for 2 min. Solid calcium chloride was added to the homogenate to give a final concentration of 10 mM. After standing in the cold for 20 min, the suspension was

IMMUNOREACTIVE SUCRASE-ISOMALTASE COMPLEX

3. Separation of villus and crypt cells from adult rat intestine. Separation of villus and crypt cells was prepared either by horizontal sectioning of frozen intestinal mucosa in a cryostat (3) or incubating intestinal mucosa with calcium and magnesium-free phosphate buffered saline containing 1.5 mM EDTA and 0.5 mM dithiothreitol (14).

For the horizontal sectioning, a small piece of jejunum, 5 mm square, was cut out, rinsed with cold saline and slit open on a filter paper with the villi pointing upwards. The tissue was frozen within less than one minute. The frozen tissue block was then attached to a chuck in a cryostat and cut into 20μ sections with the microtome (TISSUE-TEK II) (3). Every fifth section was attached to a microscope slide for examination after staining with toluidine blue. The intermediate sections were combined and homogenized with 1.0 ml of 10 mM potassium phosphate buffer (pH 7.0) for enzyme assay. On the other hand, isolated intestinal cell preparations were made according to the method of Weiser (14). A segment of jejunum, about 40 cm from the ligament of Treiz, was removed and the epithelial cell surface was rinsed three times with 6 ml of a cold saline through vinyl tubing tied at the ends of the segment. The jejunal segment was then filled with 5 ml of calcium and magnesium-free phosphate buffered saline containing 1.5 mM EDTA and 0.5 mM dithiothreitol (pH 7.3), and incubated for 15 min at 37°C under gentle agitation in a calcium and magnesium-free phosphate buffered saline. This solution was discarded and replaced by 5 ml of the same solution. By a series of successive incubations for 4, 2, 2, 3, 4, 5, 7, 10 and 15 min, sequential fractions of isolated epithelial cells from villus tip to lower villus and crypt cell areas were obtained. The different cell fractions were centrifuged at 900×g for 5 min. The precipitate was washed twice with calcium and magnesium-free phosphate buffered saline. Each final precipitate was suspended in 1.0 ml of 10 mM potassium phosphate buffer (pH 7.0) and homogenized. Smears of cell suspensions were stained with toluidine blue after fixation in 10% formalin.

4. Solubilization of sucrase-isomaltase complex by papain. The brush-border membrane suspension (0.5 ml) was incubated with 0.05 mg papain and 0.125 mg cysteine-HCl for 90 min at 37°C. For solubilization of sucrase-isomaltase complex from the homogenate of isolated cell suspension, 0.5 ml of the homogenate (ca. 15 mg protein/ml) was incubated with 0.15 mg papain and 0.225 mg cysteine-HCl for 90 min at 37°C. After papain treatment, the incubation mixture was dialyzed against 10 mM potassium phosphate buffer (pH 7.0) for 18 hr and centrifuged at 105,000×g for 60 min. The supernatant was used for the enzyme assay and the quantitative determination of sucrase-isomaltase complex content.

5. Single radial immunodiffusion. Immunoreactive sucrase-isomaltase com-
plex solubilized by papain from the brush-border membranes and the homogenate of isolated cell suspensions was determined by single radial immunodiffusion as described by Mancini et al. (15). The antiserum against rat sucrase-isomaltase complex was prepared from rabbit as previously described. The antiserum showed antigenicity for the sucrase and isomaltase moiety (16). In a 1:150 volume ratio, the antiserum was mixed with 1.2% fluid agar in barbital buffer (pH 8.6) containing 0.9% sodium chloride, 0.1% sodium azide and 0.1% Tween 80. The thickness of the gel was uniformly set at 2 mm on the immunoplate. The wells of 2 mm diameter were filled with standard solutions of sucrase-isomaltase complex or samples.

6. Sephadex G-200 column chromatography. Papain-solubilized sucrase-isomaltase complex from isolated cell suspensions was applied to Sephadex G-200 column (1.5 × 30 cm) which was equilibrated with 10 mM potassium phosphate buffer (pH 7.0) and eluted with the same buffer in the cold room. Flow rate was 4.5 ml/hr. Three-milliliter fractions were collected and aliquots were used for the enzyme assay.

7. Assay procedure. Disaccharidase activities were determined by the method of Dahlqvist (17). Sucrose and maltose were used in a final concentration of 28 mM, and isomaltose was 2.8 mM in 0.1 M sodium maleate buffer (pH 6.0). Protein concentration was determined by the method of Lowry et al. (18) using bovine serum albumin as a standard.

8. Chemicals. Papain and glucose oxidase were obtained from Worthington Biochemicals Co. Sucrose was purchased from Wako Pure Chemical Industries, Ltd. Maltose and isomaltose were kindly provided by Hayashibara Co., Ltd. Other reagents were conventional analytical grade chemicals. Sephadex G-200 was obtained from Pharmacia Fine Chem.

RESULTS

1. Developmental changes of sucrase-isomaltase complex in rat intestinal brush-border membrane

In the course of development, rat intestinal sucrase and isomaltase activities appear at the time of weaning. Our previous study on Sephadex G-200 column chromatography of papain-solubilized disaccharidase from rat intestine of different ages revealed that sucrase and isomaltase activities appeared simultaneously as a complex in the brush-border membrane 15 days after birth, and the activity ratio of sucrase to isomaltase changed thereafter (12).

In good agreement with our previous study, sucrase and isomaltase activities appeared 15 days after birth and increased rapidly until weaning; after weaning, the enzyme activities decreased. Thus, the activity ratio of sucrase to isomaltase gradually increased from 0.57 to 1.98 in the course of development (Table 1).

In order to know whether the increase in activities of sucrase-isomaltase complex may be ascribed to the synthesis of the enzyme or the activation of proenzyme, the brush-border membranes were isolated from suckling and adult rat
Table 1. Developmental changes of sucrase and isomaltase activities and contents in rat intestinal brush-border membrane.

<table>
<thead>
<tr>
<th>Age</th>
<th>12 days old</th>
<th>15 days old</th>
<th>18 days old</th>
<th>21 days old</th>
<th>25 days old</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-I, sucrase activity</td>
<td>ND</td>
<td>1.33 ± 0.48</td>
<td>32.5 ± 8.3</td>
<td>77.8 ± 2.1</td>
<td>126 ± 10\textsuperscript{a}</td>
<td>77.0 ± 1.7\textsuperscript{b}</td>
</tr>
<tr>
<td>Isomaltase activity</td>
<td>ND</td>
<td>2.29 ± 0.65</td>
<td>33.8 ± 8.2</td>
<td>83.1 ± 5.2</td>
<td>89.8 ± 3.7\textsuperscript{c}</td>
<td>39.1 ± 2.1\textsuperscript{d}</td>
</tr>
<tr>
<td>Content of S-I</td>
<td>ND</td>
<td>ND</td>
<td>47.1 ± 9.6</td>
<td>86.3 ± 9.0</td>
<td>134 ± 3.7\textsuperscript{e}</td>
<td>105 ± 9.4\textsuperscript{f}</td>
</tr>
<tr>
<td>Sucrase activity</td>
<td>—</td>
<td>0.57 ± 0.06</td>
<td>1.04 ± 0.20</td>
<td>0.95 ± 0.48</td>
<td>1.40 ± 0.10\textsuperscript{g}</td>
<td>1.98 ± 0.08\textsuperscript{h}</td>
</tr>
<tr>
<td>Isomaltase activity</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The activities and contents of sucrase-isomaltase complex were determined in the supernatants of brush-border membrane solubilized by papain. The values are expressed as means ± standard errors of five preparations. Enzyme activity, \( \mu \text{mol-substrate-hydrolyzed/mg-prot/hr} \); contents of S-I, \( \mu \text{g/mg-prot} \); S-I, sucrase-isomaltase complex; ND, not detected. Significantly different at \( p < 0.05, e-f; p < 0.01, a-b, c-d, g-h \).
intestines, and their content of immunoreactive sucrase-isomaltase complex was determined in papain-solubilized fraction by single radial immunodiffusion using specific antiserum against sucrase-isomaltase complex. The immunoreactive sucrase-isomaltase complex increased in parallel with the activities until weaning, however, higher amounts of the immunoreactive sucrase-isomaltase complex were found in the adult rat as compared with their activities (Table 1).

2. Changes of sucrase-isomaltase complex in epithelial cell migration from the crypt up to the villus tip

The epithelial cell of the intestine begins as an undifferentiated cell at the base of the crypt and migrates to the villus tip accompanied by functional changes. Sucrase activity in the intestinal epithelial cell is subjected to changes during migration from crypt to villus tip (3). Therefore, villus and crypt cells were separated from adult intestinal mucosa and the changes of sucrase-isomaltase complex during cell migration from the crypt to the villus tip was observed.

In the initial experiment, the distribution of sucrase and isomaltase activities was observed in tissue sections serially sliced from the villus tip to the crypt. As shown in Fig. 1, the distribution of sucrase and isomaltase activities were similar, with high activities located in the apical and middle villus. These activities decreased in the basal part of the villus and were much lower in the crypt.

![Distribution of sucrase and isomaltase activities in the homogenates of serial sections from the villus to the crypt in adult rat jejunum. Every fifth section of 20 μ thickness was prepared for microscopic analysis. The intermediate four sections were combined and homogenized for enzyme assay. The values are expressed as means ± standard errors of four animals.](J. Nutr. Sci. Vitaminol.)
Fig. 2. Light microscopy of cross-sections from the jejunum in adult rat. The sectioned tissues were stained with toluidine blue. a, upper villus; b, middle villus; c, crypt.

Fig. 3. Distribution of sucrase and isomaltase activities and contents in isolated epithelial cells from adult rat jejunum. The percentage of cells isolated in each successive cell fraction was determined by the proportion of cell protein isolated in a given fraction. Activities and contents of sucrase-isomaltase complex were determined in the supernatants of the cell homogenate solubilized by papain. The values are expressed as means ± standard errors of five animals.

Vol. 26, No. 2, 1980
Histologic observation revealed that the tips of the villi were more circular in cross-section, the middle of the villi were long and the crypts were like islets (Fig. 2). This method does not give sufficient villus cells or crypt cells to determine immunoreactive sucrase-isomaltase complex or to subject them to Sephadex G-200 column chromatography.

Therefore, the epithelial cells were isolated according to the method of Weiser (14). This method appeared to separate cells similar to the villus to crypt gradient as does the sectioning method (Fig. 3). Sucrase and isomaltase activities were high in the upper villus and low in the crypt. Furthermore, histologic examination showed that villus and crypt cells were well separated. As shown in Fig. 4, two types of epithelial cells were observed. Large columnar cells with brush-borders were from the upper or middle villus and smaller rounded cells were from the crypt.

Sucrase-isomaltase complex was solubilized by papain from these isolated epithelial cell homogenates. The immunoreactive sucrase-isomaltase complex was determined in the papain-solubilized fraction. The immunoreactive sucrase-isomaltase complex was increased with the migration of epithelial cells from the crypt to the villus tip. However, it is interesting to note that a significant amount of protein with immunoreactivity against anti-sucrase-isomaltase antibody was detected in the crypt cells in spite of low sucrase and isomaltase activities (Fig. 3).

3. **Sephadex G-200 column chromatography of sucrase-isomaltase complex in the upper villus and crypt cells**

In order to examine whether sucrase exists as a complex in the crypt cells, the
chromatographic behavior on Sephadex G-200 column of papain-solubilized fractions was observed in the isolated cells from the upper villus and crypt. Chromatographic profiles of the upper villus cells and crypt cells revealed that the main maltase activity was eluted at the void volume of the column, while sucrase and isomaltase activities were retained on Sephadex and eluted later, suggesting that sucrase and isomaltase exist as a complex in the crypt cells as well as in the villus cells (Fig. 5). On the other hand, free isomaltase, which was observed in the suckling rat intestine (12), was not detected. The activity ratio of sucrase to isomaltase did not show any significant changes between villus and crypt cells.

Fig. 5. Elution patterns on Sephadex G-200 column chromatography of sucrase-isomaltase complex solubilized by papain in upper villus and crypt cells of adult rat jejunum. Papain-solubilized sucrase-isomaltase complex (0.5 ml) from upper villus or crypt cells in adult rat jejunum were applied to Sephadex G-200 column (1.5 × 30 cm) equilibrated with 10 mM potassium phosphate buffer (pH 7.0) and eluted by the same buffer. Three-milliliter fractions were collected and aliquots were used for the enzyme assay. a, upper villus; b, crypt.
In the present paper, the appearance of immunoreactive sucrase-isomaltase complex was observed in rat small intestine during postnatal development and maturation along the villus-crypt axis by single radial immunodiffusion using specific antiserum against sucrase-isomaltase complex.

In the course of development, the activities of sucrase-isomaltase complex were increased in parallel with the increase in immunoreactive sucrase-isomaltase complex until weaning. This finding suggests that the increase in activities of sucrase-isomaltase complex until weaning will be ascribed to the synthesis of active enzyme or the synthesis of inactive proenzyme followed by rapid conversion to active enzyme.

Dubs et al. reported the presence of proenzyme of active sucrase in suckling rabbit intestine and in the crypt cells of adult rat intestine by immunofluorescence microscopy (10). Inability to detect immunoreactive sucrase-isomaltase complex prior to the appearance of the enzyme activity in rat intestinal brush-border is probably not solely due to the sensitivity of the method, but due to the species differences or different intracellular localization of the proenzyme. In the suckling animal, some digestive enzymes exist in a soluble form and most other intestinal proteins are also altered during development (2). Therefore, it is still possible that inactive sucrase-isomaltase complex exists in the epithelial cell except for brush-border membrane in suckling rat.

During development, the increase of immunoreactive sucrase-isomaltase complex was parallel to the increase of sucrase activity rather than isomaltase activity. The antiserum used could react immunochemically with the sucrase moiety and also isomaltase moiety (16), however, it is not known whether the immunoreactivity of the antiserum against both moieties is the same during developmental changes of sucrase-isomaltase complex. It is possible that some changes occur in the immunoreactivity accompanying the modification of sucrase-isomaltase complex.

On the other hand, higher amounts of the immunoreactive enzyme proteins were detected in crypt cells of adult rat intestine compared with their activities. This suggests that the inactive or low activity sucrase-isomaltase complex exists in the crypt cells of adult rat intestine as observed in those of adult rabbit intestine.

The inactive sucrase-isomaltase complex was detected immunologically in patients with sucrase-isomaltase deficiency, their siblings or normal human jejunal crypts (19–21). The absence of enzyme activity in the patient could be explained by a mutation of the structural gene. However, it is thought that inactive enzyme, which is observed in the crypt cells of adult rat intestine, is mildly different from active enzyme. The rabbit sucrase-isomaltase complex appears to be inserted into the plasma membrane via its N-terminal region (22). Therefore, inactive enzyme will be subject to activation such as changes of integration into plasma membrane, separation of a small peptide or modification of sugar moiety in the enzyme. It is of
interest that the changes in sucrase activity of rat intestinal mucosa by sucrose feeding appeared to be initiated at the level of crypt epithelial cells (23).

On the other hand, chromatographic behavior on Sephadex G-200 column showed that sucrase and isomaltase formed a similar complex in villus and crypt cells. This indicates that sucrase and isomaltase appear as a complex in crypt cells. In the course of development, sucrase and isomaltase appear as a complex, however, the activity ratio of sucrase to isomaltase changes from 0.57 to 1.98. Furthermore, free isomaltase, which does not make a complex with sucrase, was observed in the soluble fraction of intestinal cells of suckling rat (12). Free isomaltase was not observed in the crypt cells of adult rat intestine.

From these results, it is suggested that the activation of sucrase-isomaltase complex during development is slightly different from that in the process of maturation of epithelial cells along the villus-crypt axis. However, further studies will be required to solve the activation mechanism of proenzyme and its subcellular localization except for brush-border membrane.

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REFERENCES


