DEVELOPMENTAL CHANGES IN THE SUCRASE-ISOMALTASE COMPLEX IN RAT INTESTINAL MUCOSA

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Summary Developmental changes in sucrase-isomaltase complex formation were investigated in intestinal mucosal homogenates and brush border membranes of 15-day-old, 18-day-old and adult rats using Sephadex G-200 column chromatography and polyacrylamide disc gel electrophoresis. Disaccharidases were solubilized by papain treatment. The molecular weight of the complex did not change during development, however, the activity ratio of sucrase to isomaltase increased during development. Furthermore, a significant amount of free isomaltase, which was probably not to be derived from intestinal brush border membrane, was detected before the weanling.

The brush border membranes of intestinal cells contain disaccharidases, such as sucrase [EC 3.2.1.48], isomaltase [EC 3.2.1.10] and maltase [EC 3.2.1.20], which are involved in the terminal digestion (1, 2) and the absorption of sugar (3). Among these enzymes, sucrase and isomaltase have been isolated as a complex form from rabbit (4), human (5) and rat (6, 7). It is reported that this enzymatic complex has a close relationship with sugar transport across the brush border membranes (8) and has a role as specific carrier of sugar in the artificial lipid membrane (9). However, it is not well understood how sucrase and isomaltase are integrated into the brush border membranes.

It is well known that sucrase and isomaltase appear at the time of weanling in mammals (10). Therefore, in order to gain further understanding of the formation of sucrase-isomaltase complex, the developmental changes of this enzyme complex were observed in the rat intestinal mucosal homogenate and brush border membranes using Sephadex G-200 column chromatography and polyacrylamide disc gel electrophoresis.
MATERIALS AND METHODS

Homogenate of intestinal mucosa. Intestinal mucosa were obtained from rats of the Wistar strain. Adult male rats, weighing 200 g, and young weanling rats were fed laboratory chow (Oriental Yeast Co.) for a week and used for the experiment. Water and food were given ad libitum. For the study of disaccharidases in the developing small intestine, the litters were reduced at birth to 10–12 and intestines were taken from 15-, 16-, 17-, 18-, 19-, 20- and 22-day-old rats. The room light was turned on at 0600 hour and turned off at 1800 hour. Rats were killed by decapitation between 1300 and 1500 hour. Intestinal mucosa was homogenized with 4 volumes of 10 mM pottasium phosphate buffer (pH 7.0) to give 20% homogenate, and stored at −20°C until use. An aliquot of the homogenate was used for the solubilization of disaccharidases by papain treatment and the assay of disaccharidases activities.

Preparation of brush border membrane. Preparation of the brush border membranes was carried out according to the method of KESSLER et al. (11). Mucosal scrapings from 16 suckling rats of 15-day-old or 3 adult rats 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 10 mM. After standing in the cold for 15 min, the suspension was centrifuged at 3,000×g for 15 min. The pellet was discarded. The supernatant was centrifuged at 27,000×g for 30 min. The pellet was then resuspended in 10 ml of 50 mM mannitol in 10 mM Tris-HCl buffer (pH 7.1) and centrifuged once more at 27,000×g for 30 min. This pellet, which contained almost pure brush border membrane, was resuspended in 2 ml of 10 mM pottasium phosphate buffer (pH 7.0).

Solubilization of disaccharidases by papain treatment. Five milliliters of the homogenate from 15-, 18-day-old and adult rat was incubated with 1.6 mg papain and 4.0 mg cysteine-HCl for 90 min at 37°C. For solubilization of disaccharidases from brush border membrane, 2 ml of brush border membrane suspension (ca. 9 mg protein/ml) was incubated with 0.3 mg papain and 0.75 mg cysteine-HCl for 90 min at 37°C. After papain treatment, the incubation mixture was centrifuged at 105,000×g for 60 min. Supernatants were used as a papain solubilized disaccharidases.

Sephadex G-200 column chromatography. Papain solubilized disaccharidases (1.5 ml) were applied to Sephadex G-200 column (1.5×30 cm) which was equilibrated with 10 mM pottasium phosphate buffer (pH 7.0) and eluted with the same buffer in the cold room. Flow rate was 4.5 ml/hour. Three milliliter fractions were collected and their aliquots were used for the enzyme assay.

Polyacrylamide gel disc electrophoresis. Polyacrylamide gel disc electrophoresis of the eluates of Sephadex G-200 column chromatography was carried out essentially according to the method of DAVIS (12). The same sample was run at three different gel concentrations, viz, 4, 6, and 8% in acrylamide at pH 8.3.
in Tris-glycine buffer. In the 15-day-old rat sample, three different gels were run simultaneously for each gel concentration. After electrophoresis, the gels were frozen. The frozen gels were sliced into 2 mm pieces. Sliced gels of the 15-day-old rat sample were soaked in 0.1 ml redistilled water for 2 hr at room temperature and used for one enzyme assay. On the other hand, sliced gels of adult rat sample were soaked in 0.3 ml redistilled water for 2 hr at room temperature and each 0.1 ml was used for each enzyme assay. Molecular weight analysis was carried out according to the method of HEDRICK et al. (13). For the molecular weight standardization of gel, ovalbumin, albumin, γ-globulin and apoferritin were used. Samples were applied on the gels with glycerol. Usually, 0.1 ml of sample was applied on each gel.

**Assay procedure.** Disaccharidases activities were determined by the method of DAHLQVIST (14). Substrate concentration was 28 mM in sucrose, maltose and lactose in 0.1 m sodium maleate buffer (pH 6.0), 2.8 mM in isomaltose. Protein concentration was determined by the LOWRY method (15) using bovine serum albumin as a standard.

**Chemicals.** Papain and glucose oxidase were obtained from Worthington Biochemicals Co. Acrylamide was from Tokyo Kasei, Ltd. Standard marker proteins used for the determination of molecular weight were products of Schwarz/Mann. Sucrose and lactose were from Wako Pure Chemical Industries, Ltd. Maltose and isomaltose were kindly provided by Hayashibara, Co., Ltd.

**RESULTS AND DISCUSSIONS**

In good agreement with the findings of other investigators (10, 16), sucrase, isomaltase and maltase activities of intestinal homogenate were increased at the time of weanling, while lactase activities were decreased (Fig. 1). Sucrase, isomaltase and maltase of young and adult rats showed a little low values compared with those of 22-day-old rats. This must be due to the circadian changes corresponding to dietary intake (17) which became apparent after weanling. Day time disaccharidases activities are lower compared with night time activities (18).

It is known that a sucrase-isomaltase complex is retained by Sephadex G-200 owing to a substrate-enzyme interaction mainly involving its isomaltase site (4). Therefore, in order to know that the upsurge of disaccharidases activities accompany the changes in sucrase-isomaltase complex, the chromatographic behavior on Sephadex G-200 column chromatography of papain solubilized disaccharidases was observed in various stages of developing rats. The intestinal mucosal homogenate of 15-, and 18-day-old and adult rats, taken from before, in the middle of and after the upsurge of disaccharidases activities, were treated with papain. Recovery of disaccharidases activities in papain soluble fraction was about 70% and was not changed in three preparations. Chromatographic
Fig. 1. Changes of rat intestinal disaccharidases activities in development. Disaccharidases activities were assayed in the intestinal homogenate obtained from rats of various ages. a, sucrase; b, isomaltase; c, maltase; d, lactase. The values are expressed as means ± standard errors of 5 or 8 rats.

profiles revealed that the main maltase activities were excluded at the void volume of the column, while sucrase and isomaltase activities were retained on Sephadex and eluted later at total volume (Vt) of the column. Some maltase activities were detected with sucrase and isomaltase activities. Furthermore, a significant amount of isomaltase activity was detected at void volume of the column in the 15-day-old rats. During growth, this fast emerging isomaltase probably increases, however, the isomaltase associated with sucrase increases much more, so that the proportion of isomaltase activity at void volume of the column decreased rapidly with age. In the 18-day-old rats, some isomaltase activity was detected clearly at void volume. In the adult rat essentially all isomaltase activities were retained on Sephadex and
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Fig. 2. Elution patterns on Sephadex G-200 column chromatography of rat intestinal disaccharidases solubilized by papain. Papain solubilized disaccharidases (1.5 ml) from 15-, 18-day-old and adult rat intestines were applied to Sephadex G-200 column (1.5×30 cm) equilibrated with 10 mM potassium phosphate buffer and eluted by the same buffer. 3 ml fractions were collected and aliquots were used for the enzyme assay.

![Elution patterns on Sephadex G-200 column chromatography](image)

eluted with sucrase (Fig. 2).

On the other hand, the activity ratio of sucrase to isomaltase was calculated from the elution profiles of Sephadex G-200 column chromatography (Fig. 3). In adult rat, the ratio was approximately 2.5 and did not change during elution (fraction number 12 to 25). In 15-day-old rat, the ratio was changed gradually from 0.4 to 1.2 (fraction number 12 to 25), but the ratio was not increased to that of adult rat.

In order to know that the changes in activity ratio of sucrase to isomaltase are due to the changes in molecular size, molecular weight of disaccharidases was determined by electrophoresis. From the results of Furgason plotts (13), the molecular weight of maltase eluted at void volume was estimated about 400,000 daltons. Sucrase and isomaltase which are retained on Sephadex was estimated
as about 200,000 and has maltase activity. However, it is interesting to note that there exists isomaltase and maltase which have not formed complexes with sucrase in 15-day-old rat. Their molecular weight was about 110,000 daltons (Table 1).

In order to know whether this isomaltase is derived from brush border membranes, brush border membranes were prepared from 15-day-old and adult rat intestine and their chromatographic behaviour on Sephadex G-200 was observed. As shown in Fig. 4, the elution profile of adult rat was essentially same as that of homogenate, however, fast emerging isomaltase was not detected in 15-day-old rat. Therefore, this isomaltase is not from brush border membranes.

In sucrase-isomaltase malabsorption, sucrase activity is totally absent, but some isomaltase activity is still present which is not attributed to isomaltase activity of the sucrase-isomaltase complex because of its different heat resistance (19) and the absence of both protein bands of sucrase-isomaltase complex in

Table 1. Molecular weight of rat intestinal disaccharidases. Fraction I denotes the fractions (8–10) and Fraction II denotes the fractions (18–22) on Sephadex G-200 column chromatography. The data were expressed as mean±S.E. of three determinations. Each disaccharidase activity was assayed respectively in different gels.

|                | 15-day-old rat | Adult rat |
|----------------|----------------|-----------|
|                | Fraction I     | Fraction II | Fraction I     | Fraction II |
| Sucrase        | 220,000±13,000 |            | 195,000±11,000 |
| Isomaltase     | 115,000±8,000  | 195,000±8,000 | 195,000±11,000 |
| (15-day old)   | 112,000±7,000  | 217,000±13,000 | 195,000±11,000 |
| Maltase        | 415,000±20,000 |            | 388,000±5,000  |
Fig. 4. Elution patterns on Sephadex G-200 column chromatography of rat intestinal disaccharidases solubilized from brush border membrane by papain. The enzyme assay is the same in Fig. 2.

polyacrylamide gel electrophoresis of biopsy samples from these patients (20). This isomaltase activity is, therefore, thought to be due to one of glucoamylases.

Further work is necessary to establish whether the fast emerging isomaltase of molecular weight 110,000 is identical with the glucoamylase, or whether it is identical with but appearing earlier than the isomaltase moiety of the sucrase-isomaltase complex.

Semenza suggested that sucrase-isomaltase complex is composed of hydrophobic part, presumably embedded into the lipid bilayer of the membrane and anchoring the protein at the membrane, and a much larger hydrophilic part carrying the sugar moiety and hydrolytic active sites (21). At least, it could be assumed from our present results that sucrase and isomaltase appear as a complex in the membranes and after the integration of the complex into the membrane, the complex will be modified. These and other possibilities are presently being investigated.

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