The Influence of Hardly Digestive Starch Granules on Sucrase and Isomaltase in Small Intestinal Mucosa of Rats

Shuzo FUJITA and Hidetsugu FUWA¹

*Department of Food and Nutrition, Faculty of Science of Living,
Osaka City University, Sumiyoshi-ku, Osaka 558, Japan*

(Received June 3, 1983)

Summary The effects of potato-starch granules (PSG) on sucrase and isomaltase activities in the small intestinal mucosa of rats were examined by gel filtration on a Sephacryl S-300 column. Young and adult rats were administered the diet containing 50% of either PSG or pregelatinized potato starch (PPS) as a control for 7 days. Mucosal homogenates were prepared from the small intestine and separated into their component disaccharidases on the column. The sucrase activity, increased by feeding young rats on the PSG diet, was detected in the sucrase-isomaltase complex by changing the activity ratio of the two enzymes. In adult rats, however, the complex seemed to be mature and the increased sucrase moiety was newly combined with a free isomaltase moiety and comprised a portion of the complex. These changes were observed not only in soluble disaccharidases but also in membrane-bound ones. Furthermore, there was a remarkable change of sucrase activity in the distal portion of the small intestine rather than in the proximal.

Key Words starch granule digestion in rats, potato-starch granules (PSG), sucrase, isomaltase, sucrase-isomaltase complex, Sephacryl gel-filtration

The susceptibility of starch granules to digestion by amylase depends on the source of the starch and the enzyme. Starch granules of roots and tubers are generally more resistant to the action of amylase than those of cereals (1–3). Potato-starch granules (PSG) are the most resistant to amylase among various starch granules. We investigated the influence of hardly digestive starch such as PSG on digestive tracts and enzymes of rats, and showed that α -amylase [EC 3.2.1.1.] activity in pancreas was significantly decreased by feeding rats on a PSG diet and that intestinal sucrase [EC 3.2.1.48] activity was significantly increased, but that intestinal maltase [EC 3.2.1.20] and isomaltase [EC 3.2.1.10] activities scarcely changed (4–6). These changes were also observed when feeding rats on a diet

¹ 藤田修三, 不破英次

containing dietary fiber of high water-holding capacity, for example Konbu, a kind of sea vegetable (7).

Among intestinal disaccharidases of mammals, isomaltase exists both in a free form and as a complex with sucrase (8-10). However, little is known about whether the sucrase activity, increased by feeding rats on a PSG diet, actually operates *via* the sucrase-isomaltase complex and plays a role on the terminal digestion and absorption of the starch. This paper describes the fractionation of small intestinal disaccharidases by gel filtration on a Sephacryl S-300 column, to obtain detailed information on sucrase and isomaltase activities in the intestinal mucosa of rats.

EXPERIMENTAL

1. *Animals and diets.* Experimental diets were administered to male Sprague-Dawley rats, 4 and 8 weeks of age, for 7 days. Rats were housed in individual stainless-steel wire cages in a temperature-regulated ($22 \pm 2^\circ\text{C}$) and light-controlled (light from 08:00 to 20:00) room. One hundred grams of experimental diet (PSG diet) contained 25 g of casein, 50 g of PSG and 9 g of pre-gelatinized potato starch (PPS) to make a diet dumpling with water, 5 g of corn oil, 5 g of cellulose, 5 g of salt mixture, 0.85 g of water-soluble vitamins, 10 mg of all *rac*- α -tocopheryl acetate, 600 IU of retinyl palmitate and 60 IU of ergocalciferol (4). The control PPS diet contained 50 g of PPS instead of the PSG in the PSG diet. Animals were given water and the diet in the form of dumplings *ad libitum*. After experimental period, rats were anesthetized in an ether chamber and killed. The small intestine was removed and its mucosa was collected by scraping

2. *Preparation of soluble and membrane-bound disaccharidases.* Disaccharidases were prepared by the method of Yamada *et al.* (11). The intestinal mucosa was homogenized with 4 volumes of 10 mM sodium phosphate buffer, pH 7.2. To separate soluble enzymes from insoluble membrane-bound ones, the homogenate was centrifuged at $105,000 \times g$ for 1 h. The supernatant was used since it contained soluble disaccharidases.

A preparation of the brush-border membrane was obtained by the method of Kessler *et al.* (12). The $105,000 \times g$ precipitate was suspended in 30 volumes of ice-cold 50 mM mannitol dissolved in 2 mM Tris-HCl buffer, pH 7.1, and homogenized in a glass homogenizer with a Teflon pestle for 2 min. Solid calcium chloride was added to the homogenate to give a final concentration of 10 mM. After standing in an ice bath for 20 min, the suspension was centrifuged at $3,000 \times g$ for 15 min; the supernatant was centrifuged at $27,000 \times g$ for 30 min. The pellets were then resuspended in 50 mM mannitol dissolved in 10 mM Tris-HCl buffer, pH 7.1, and centrifuged at $27,000 \times g$ for 30 min. The resultant pellets were resuspended in 2 ml of 10 mM sodium phosphate buffer, pH 7.2 (brush-border membrane suspension) and the suspension was incubated with 1.6 mg papain and 4.0 mg cysteine-HCl for 90 min at 37°C . After papain treatment, the incubation mixture was centrifuged at $105,000 \times g$ for 1 h. The supernatant was used for its membrane-bound disaccha-

ridase content.

3. *Sephacryl S-300 gel filtration.* Either soluble or membrane-bound disaccharidase (1.0 ml) was applied to a Sephacryl S-300 column (1.5 × 78 cm) equilibrated with 10 mM sodium phosphate buffer, pH 7.2, in a cold room. The same buffer was used for elution with a fraction volume of 2.5 ml and flow rate of 14 ml per h.

4. *Assay procedures.* Disaccharidase activities were determined by the method of Dahlqvist (13). Substrate concentration was 25 mM respectively of isomaltose, lactose, maltose and sucrose in 50 mM sodium acetate buffer, pH 6.0. One unit of enzyme activity was defined as the amount of enzyme hydrolyzing 1 μ mol substrate in 1 h at pH 6.0 and 37°C. "Glucoamylase" activity was measured by the method of Schlegel-Haueter *et al.* (14). One unit of enzyme activity was defined as the amount of enzyme releasing 1 μ g of glucose from substrate in 1 h.

RESULTS

Mucosal disaccharidases on a Sephacryl S-300 column

Sephacryl is a covalently cross-linked allyl dextran with *N,N'*-methylene bisacrylamide and has rigid and highly stable matrices. Therefore, the gel may have the advantage of rapid separation of maltase, isomaltase and sucrase because of the higher affinity of the isomaltase to α -1,6-glucosidic linkages in dextran such as Sephadex than that of the other enzymes.

As shown in Fig. 1, gel filtration of the intestinal preparations on the Sephacryl S-300 column revealed a major peak containing a sucrase-isomaltase complex accounting for about 90% of the total activity of sucrase and about 55% of the total

Fig. 1. Elution patterns of chromatography on a column of Sephacryl S-300 of several disaccharidases and "glucoamylase" of the small-intestinal mucosa in adult rats. —, isomaltase; ---, sucrase; - · -, maltase; - · · -, lactase; · · · · ·, "glucoamylase" activities.

Table 1. Chromatography of soluble and membrane-bound disaccharidases in the small-intestinal mucosa on feeding young and adult rats on either PPS or PSG (Sephacryl S-300 column).

	Isomaltase activity ^a			S-I ratio ^b	Enzyme activity (μ mol hydrolyzed substrate/min/mg protein)	
	Fraction 1	Fraction 2	fr. 2/fr. 1		Sucrase ($\times 10^{-2}$)	Isomaltase ($\times 10^{-1}$)
(Soluble disaccharidases in young rats)						
PPS (<i>n</i> =5)	67.2 $\pm$ 2.5 ^c	32.8 $\pm$ 2.5	0.48 $\pm$ 0.06	0.99 $\pm$ 0.10	3.04 $\pm$ 0.46	1.22 $\pm$ 0.45
PSG (<i>n</i> =5)	67.1 $\pm$ 4.8	32.9 $\pm$ 4.8	0.50 $\pm$ 0.11	0.89 $\pm$ 0.07	4.11 $\pm$ 0.16 ^d	0.96 $\pm$ 0.17
(Soluble disaccharidases in adult rats)						
PPS (<i>n</i> =4)	56.8 $\pm$ 4.6	43.2 $\pm$ 4.6	0.77 $\pm$ 0.14	0.92 $\pm$ 0.18	2.30 $\pm$ 0.35	1.09 $\pm$ 0.21
PSG (<i>n</i> =4)	69.9 $\pm$ 4.2	30.1 $\pm$ 4.2	0.44 $\pm$ 0.09 ^d	0.96 $\pm$ 0.13	4.73 $\pm$ 0.21 ^d	0.92 $\pm$ 0.09
(Membrane-bound disaccharidases in adult rats)						
PPS (<i>n</i> =2)	51.7	48.3	0.93	0.96		
PSG (<i>n</i> =2)	70.7	29.4	0.41	0.93		

^a Fractions 1 and 2 represent isomaltase fractions eluted respectively faster and slower on Sephacryl column. See Fig. 1. ^b Activity ratio of sucrase to fraction 1 of isomaltase. ^c Values are expressed as means $\pm$ SD. ^d Significantly different from the PPS group at $p < 0.01$.

Table 2. Chromatography of soluble disaccharidases in upper and lower small-intestinal mucosa on feeding adult rats on either PPS or PSG (Sephacryl S-300 column).

	Isomaltase activity ^a			S-I ratio ^a	Enzyme activity (μ mol substrate hydrolyzed/min/mg protein)	
	Fraction 1	Fraction 2	fr. 2/fr. 1		Sucrase	Isomaltase
PPS Total (<i>n</i> = 3)	55.5	44.5	0.80	1.05	2.22×10^{-2}	1.02×10^{-1}
Upper (<i>n</i> = 3)	73.3	26.7	0.36	1.09	1.72×10^{-2}	0.54×10^{-1}
Lower (<i>n</i> = 3)	48.7	51.3	1.05	0.96	3.99×10^{-2}	1.73×10^{-1}
PSG Total (<i>n</i> = 3)	65.1	34.9	0.54	0.97	4.83×10^{-2}	0.82×10^{-1}
Upper (<i>n</i> = 3)	74.4	25.6	0.34	1.05	5.14×10^{-2}	0.59×10^{-1}
Lower (<i>n</i> = 3)	62.4	37.6	0.60	1.30	5.08×10^{-2}	1.00×10^{-1}

^a See legend to Table 1.

activity of isomaltase in adults rats. Other peaks of sucrase and isomaltase were also detected. Two peaks having maltase activity were detected from chromatography as well as both chromatography on a Sephadex G-200 column (15) and acrylamide-gel electrophoresis (10). The heavier maltase was associated with "glucoamylase" activity. Lactase was eluted in between the two maltases.

Elution profiles of sucrase and isomaltase of young rats, on the Sephacryl S-300 column

Chromatographic behavior of the soluble disaccharidases on the Sephacryl column was followed in order to determine whether the sucrase activity, increased by feeding rats on the PSG diet, exists as a complex with isomaltase. The elution profile of isomaltase on feeding rats on the PSG diet was similar to that of the control. The sucrase activity, increased by the substitution of PSG for PPS in the PPS diet, was then detected in the sucrase-isomaltase complex. The activity ratio of isomaltase to sucrase in the complex fraction decreased from 0.99 to 0.89, while the ratio of total activity of isomaltase to that of sucrase was decreased from 1.45 to 1.05 as reported previously (6) (Table 1).

Elution profiles of sucrase and isomaltase of adult rats, on the Sephacryl S-300 column

In the case of the soluble disaccharidases of adult rats, the total activity ratio of isomaltase to sucrase decreased from 1.54 to 1.15 by the substitution of PSG for PPS in the PPS diet as well as in the case of young rats. However, the activity ratio of "slow" isomaltase (fraction 2) to "fast" isomaltase (fraction 1) decreased from 0.77 to 0.44 by feeding the rats on the PSG diet (Table 1). The sucrase-isomaltase ratio of the complex fraction was almost the same for the two groups. This means that feeding the rats on the PSG diet promotes the formation of the sucrase-isomaltase complex in the small-intestinal mucosa of rats. The elution profiles of the membrane-bound enzymes were also similar to those of the respective soluble enzymes.

To obtain information on isomaltase and sucrase activities in each part of the small intestine, the tissue of adult rats was divided into upper and lower portions. The mucosal homogenates of each portion were then applied to the column. In the upper portion, probably corresponding to duodenum and jejunum, the activity ratio of fraction 2 (a free form) to fraction 1 (a complex with sucrase) of isomaltase was similar in both the PSG and the control groups. However, in the lower portion, probably the ileum, the ratio was different between the PSG (0.60) and the control (1.05) (Table 2). The results suggested that the change of sucrase activity by feeding animals on the PSG diet occurred in the ileum rather than in the jejunum.

DISCUSSION

Sephacryl S-300 gel filtration is a useful device for taking an overall view of the

elution profiles of several disaccharidases, especially isomaltase, offering a considerable reduction in time compared with Sephadex G-200 gel filtration. Moreover, isomaltase activity was eluted in order of molecular weight by the Sephacryl gel filtration because of the lesser content of dextran in the gel and the lower affinity to isomaltase than with Sephadex.

We reported in our previous paper (6) that the intestinal sucrase activity was increased by feeding young rats on the PSG diet. Our present study shows that the increased sucrase activity was detected in the sucrase-isomaltase complex and that some differences in the complex were observed between young and adult rats. Namely, from the elution profile, activity ratio of sucrase to isomaltase was decreased by feeding young rats on the PSG diet though the ratio did not change by feeding adult rats on the experimental diet.

Isomaltase activity was separated into two fractions, fraction 1 (a complex with sucrase) and fraction 2 (a free form), as shown in Fig. 1 and the activity ratios of fraction 1 to fraction 2 of young and adult rats fed on the PPS diet were 0.48 and 0.77, respectively (Table 2). Namely, isomaltase of adult rats consists of a relatively greater amount of the free form than is the case with young rats. The ratio scarcely changed by feeding young rats on the PSG diet. However, it was decreased from 0.77 to 0.44 by feeding adult rats on the diet. The results suggest that the sucrase activity, increased by feeding young rats on the PSG diet, may be combined with that of the pre-existing sucrase-isomaltase complex without changing the free isomaltase activity or may result from the activation of the inactive sucrase moiety contained in the complex. While the sucrase activity, increased by feeding adult rats on the experimental diet, was combined with the free isomaltase activity and resulted in a newly formed sucrase-isomaltase complex.

We cannot answer at present the question as to whether the increased sucrase activity is involved in the terminal digestion and absorption of potato-starch granules or not. However, a preliminary experiment on the action of disaccharidases fractionated by Sephacryl gel filtration on an α -limit dextrin by pancreatic α -amylase showed that the sucrase-isomaltase complex and the maltase fraction having "glucoamylase" activity degraded the α -limit dextrin to a considerable extent. This suggested that an increase in the α -limit dextrinase activity, which resulted from the increased sucrase activity in ileum by the substitution of PSG for PPS in the PPS diet, may play a role in the degradation of products of intestinal α -amylolysis and may be a biochemically reasonable response for digestion and absorption of products derived by α -amylolysis of indigestible starch granules.

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