Changes of the Activity and Content of Sucrase-Isomaltase Complex in the Intestinal Mucosa during the Development of Streptozotocin-Induced Diabetes in Rats

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Summary The changes in the activity and content of sucrase-isomaltase complex (S-I) in the intestinal mucosa were studied during the development of diabetes induced by streptozotocin in rats. On days 0, 1, 3, 5, and 10 after an intraperitoneal injection of streptozotocin (70 mg/kg), the enzyme activity and the enzyme content were observed in the jejunum and ileum. Sucrase and isomaltase activities markedly increased from the 3rd day both in the jejunum and ileum, and kept increasing till the 10th day especially in the ileum. The enzyme content of S-I also increased in parallel with its activity during the development of diabetes. However, in the early stage of diabetes, sucrase activity per µg of S-I content increased both in the jejunum and ileum. Isomaltase activity per µg of S-I content increased temporarily in the ileum. These results suggest that the increase of disaccharidase activities in the early stage of diabetes induced by streptozotocin is not only due to the increase of the enzyme content, but also due to the change of the enzyme catalytic property.

Key Words sucrase-isomaltase complex, streptozotocin-induced diabetes, rat intestinal mucosa

It has been reported that experimental diabetes causes the increase of digestive and absorptive function of sugars in the brush border membranes of the intestinal epithelial cells (1–6).
We previously reported that there are two different stages of the increase of disaccharidase activities in intestinal mucosa accompanied with the development of streptozotocin-induced diabetes in rats, viz., 1) the early stage of increased specific enzyme activity before the beginning of hyperphagia (till the 5th day after streptozotocin injection), and 2) the later stage of increased total enzyme activity after the beginning of hyperphagia (the 5th day after streptozotocin injection) (7).

In the early stage of diabetes, some endocrinological effect other than food effect seems to exist (8). However, it is not clear how early the increases of disaccharidase activities appear and whether the increases of disaccharidase activities are due to the increase of enzyme content or due to the change of enzyme catalytic property in the early stage of diabetes. Therefore, we have examined the change of the enzyme activity and enzyme content of sucrase-isomaltase complex (S-I) after the injection of streptozotocin in rats.

MATERIALS AND METHODS

1) Animals. Male rats of Wistar strain, weighing 200g, were raised on standard laboratory chow diet (Oriental Yeast Co.) for a week followed by an intraperitoneal injection of streptozotocin (70mg/kg). Streptozotocin solution was prepared just before the injection with citrate buffer (pH 4.5). Control rats received the citrate buffer alone. All rats were housed in individual metabolic cages, fed ad libitum and had free access to water. On days 0, 1, 2, 3, 5, and 10 after the injection, rats were killed by decapitation between 13:00 and 15:00. Blood and 24h urine were tested for glucose concentration using the glucose-oxidase method of Dahlqvist (9). Only rats, their blood glucose concentrations were more than 250mg/100ml, were used as diabetes.

2) Intestinal homogenate. The intestine was removed immediately, cut longitudinally, rinsed with ice-cold saline and then blotted on tissue paper. Thereafter, the small intestine was divided into three segments, viz., 10cm from pylorus to duodenum, and two equal lengths of the jejunum and ileum. Jejunal and ileal mucosa was scraped with a glass slide and homogenized with 4 volumes of 10mM potassium phosphate buffer (pH 7.0) to make 20% homogenate and stored at −20°C until use. An aliquot of the homogenate was used for the assay of sucrase and isomaltase activities and S-I content.

3) Disaccharidase assay and protein determination. Disaccharidase activities were determined by the method of Dahlqvist (9). Substrate concentration was 28mM in sucrose and 2.8mM in isomaltose in 50mM sodium maleate buffer (pH 6.0). Protein concentration was determined by the method of Lowry et al. (10) using bovine serum albumin as a standard. Disaccharidase activities were expressed in specific activity (μmol-substrate-hydrolyzed/mg-protein/h). Statistical analysis was carried out by Student’s t-test.

4) Immunoassay of sucrase-isomaltase complex content. Content of S-I was determined with papain-solubilized supernatants from jejunal and ileal mucosal.
homogenate, by single radial immunodiffusion in an application (11) of the method of Mancini et al. (12). The S-I used as a standard was purified from rat intestine as previously described (13). Antiserum against rat S-I was prepared from rabbit as previously described (14).

5) Chemicals. Streptozotocin was obtained from Sigma Chemical Co. Papain and glucose oxidase from Worthington Biochemicals Co. Sucrose was purchased from Kokusan Chemical Works, Ltd. Isomaltose was kindly provided by Hayashibara Co., Ltd. Other reagents were analytical grade chemicals.

RESULTS

The changes of intestinal sucrase and isomaltase activities were observed during the development of diabetes. Sucrase and isomaltase activities increased within 3 days after the injection of streptozotocin, both in the jejunum and ileum (Fig. 1).

In order to show whether the changes in sucrase and isomaltase activities are due to the increase of enzyme amount or not, S-I content was determined immunochemically with papain solubilized supernatant of jejunal and ileal homogenate. The changes of sucrase and isomaltase activities in the papain-solubilized

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Fig. 1. Effect of streptozotocin injection on sucrase and isomaltase activities in intestinal mucosa of the rats. Each point represents mean ± SE of 5-7 animals. ——, ——, diabetic rats; ——O——, ——Δ——, control rats. a, b, c, d, e, f: Significantly different from the value of day 0, 1, 3, 5, 10 after the injection of streptozotocin or the value of day 10 control rats at p<0.05, respectively. ā, ē, ē, ē, ē, ē: Significantly different at p<0.01.
supernatant were similar to those of intestinal homogenate (Fig. 2). On the 3rd day after the injection, the marked increases of sucrase and isomaltase activities were observed both in the jejunum and ileum. On the 5th day, sucrase and isomaltase activities reached a plateau level in the jejunum, whereas those in the ileum kept increasing till the 10th day after the injection of streptozotocin (Fig. 2). S-I contents also increased after the injection of streptozotocin and the time course of the increase of S-I contents was similar to that of sucrase and isomaltase activities.

In order to examine whether there is some change of enzyme catalytic property during the development of diabetes, sucrase (S) and isomaltase (I) activities were expressed in terms of µg of S-I content. As shown in Table 1, S/S-I content in the jejunum, significantly increased on the 3rd day, whereas I/S-I content did not change during the experimental period. Thus, the activity ratio of S to I increased significantly from 1.69 to 1.96 in diabetic rats compared with control rats on the 10th day after the injection of streptozotocin (p < 0.05, Table 1). On the other hand, the increase of S/S-I content on the 3rd day in ileum was followed by the increase of I/S-I content. After the 3rd day, both S/S-I content and I/S-I content decreased. Thus, the increase of S/S-I content and I/S-I content was observed temporarily in the ileum. In the ileum, a slight but significant increase in the activity ratio of S to I
Table 1. Effect of streptozotocin injection on sucrase and isomaltase activities in comparison with the content of sucrase-isomaltase complex.

|                  | Days after injection |          |          |          |          |          |
|------------------|----------------------|----------|----------|----------|----------|----------|
|                  | 0        | 1        | 3        | 5        | 10       | 10 control |
| Jejunum          |          |          |          |          |          |          |
| S/S-I<sup>a</sup> | 0.546 ± 0.026 | 0.553 ± 0.017 | 0.623 ± 0.023* | 0.629 ± 0.036 | 0.615 ± 0.022<sup>o</sup> | 0.555 ± 0.011 |
| I/S-I<sup>b</sup> | 0.322 ± 0.017 | 0.334 ± 0.025 | 0.341 ± 0.026 | 0.326 ± 0.025 | 0.315 ± 0.018 | 0.329 ± 0.008 |
| S/I<sup>c</sup>   | 1.70 ± 0.04  | 1.68 ± 0.09  | 1.85 ± 0.07  | 1.94 ± 0.04** | 1.96 ± 0.06**<sup>d</sup> | 1.69 ± 0.05  |
| Ileum            |          |          |          |          |          |          |
| S/S-I<sup>a</sup> | 0.335 ± 0.027 | 0.342 ± 0.019 | 0.467 ± 0.027** | 0.433 ± 0.030* | 0.430 ± 0.017* | 0.423 ± 0.041* |
| I/S-I<sup>b</sup> | 0.260 ± 0.025 | 0.302 ± 0.011 | 0.382 ± 0.025** | 0.307 ± 0.027 | 0.287 ± 0.006 | 0.331 ± 0.030 |
| S/I<sup>c</sup>   | 1.30 ± 0.03  | 1.13 ± 0.04** | 1.23 ± 0.03  | 1.42 ± 0.05  | 1.50 ± 0.07<sup>o</sup> | 1.29 ± 0.03  |

<sup>a</sup>Sucrase activity (μmol-substrate/h/μg of sucrase-isomaltase complex). <sup>b</sup>Isomaltase activity (μmol-substrate/h/μg of sucrase-isomaltase complex). <sup>c</sup>Activity ratio of sucrase to isomaltase. Each value is mean ± SE of 5 animals. Significantly different from the value of day 0 at *p < 0.05 or **p < 0.01. Significantly different when comparisons were made between day 10 and day 10-control at <sup>o</sup>p < 0.05 or <sup>d</sup>p < 0.01.
was also observed in diabetic rats on the 10th day compared with control rats (1.50 vs. 1.29) (Table 1).

Moreover, it is interesting to know the difference of sucrase activity and isomaltase activity in terms of $\mu$g of S-I content between jejunum and ileum. In normal rats (10th day control), S/S-I content in the jejunum was much higher than that in the ileum ($0.56 \pm 0.01$ vs. $0.42 \pm 0.04$, $\mu$mol-substrate-hydrolyzed/h/$\mu$g of S-I content). However, there was no difference in I/S-I content between jejunum and ileum ($0.33 \pm 0.01$ vs. $0.33 \pm 0.03$). The activity ratio of S to I in the jejunum was higher than that in the ileum ($p < 0.01$, Table 1).

**DISCUSSION**

We previously reported that there were two different stages in the increase of disaccharidase activities in streptozotocin induced diabetic rats; 1) increase of specific activity before hyperphagia (for about 5 days after injection, early stage) and 2) increase of total activity as a result of the increase of mucosal weight after hyperphagia (later stage) (7). Other investigators have also reported that the increase of mucosal weight by diabetes has been caused by increased food intake (15). On the 12th day after the injection of streptozotocin, S-I content in intestinal mucosa increased in parallel with sucrase and isomaltase activities (7).

In the present study, time course of the changes of S-I content and activity in diabetic rats was observed in order to clarify how the disaccharidase activity increases in the early stage of diabetes. Marked increase of sucrase and isomaltase activities appeared on the 3rd day after the injection with the simultaneous increase of S-I content. Moreover, the change was similar between S-I content and its activity during the development of diabetes. This shows that the increase of sucrase and isomaltase activities is mainly due to the increase of the enzyme content in the early stage of diabetes as well as in the later stage of diabetes.

Olsen and Korsmo (16) reported that the increased sucrase activity in 5-day diabetes of rats was accompanied with the increased S-I content as a result of the decrease in the degradation rate of S-I. It has been also shown that pancreatic duct ligation caused a significant increase of sucrase activity (17). It is suggested that the surface of intestinal absorptive cells is being constantly remodelled by the action of pancreatic proteases (18). Interestingly, we observed that the activity ratio of S to I was higher in the jejunum than in the ileum. Recently, it was demonstrated that sucrase-isomaltase complex might be converted from a large single polypeptide by the action of pancreatic proteases (19, 20). Since insulin has been reported to stimulate the protein and digestive enzyme synthesis in exocrine pancreas (21), in the case of insulin deficiency such as streptozotocin-induced diabetes, pancreatic proteases may have some effects on the disaccharidase activities through modulating the enzyme conformation or their degradation rate.

The epithelial cell of the intestine appears as undifferentiated cell at the base of the crypt and migrates to the villus tip accompanying functional changes (22).
We recently observed that higher amount of the immunoreactive S-I protein was detected in crypt cells of adult rat intestine compared with its activity (11). In diabetic rats, S-I content and its activity also increased in crypt cells (23). These findings suggested that the increases of S-I content and its activity in diabetic rats were in some part due to the increases of enzyme synthesis and rapid conversion to active enzyme. Therefore, we examined the sucrase and isomaltase activities by the expression of hydrolyzing activity per μg of S-I content. S/S-I content increased both in the jejunum and ileum only in the early stage of diabetes, i.e., on the 3rd day, whereas I/S-I content increased only in the ileum, on the 3rd day. This suggests that there might be a change of enzyme catalytic property in the early period during the development of diabetes. The precise mechanism of the change of S/S-I and I/S-I content is not known. However, at present, we can speculate that the increase of disaccharidase activities in diabetic rats is mainly due to the increase of enzyme content, but also is associated with the change of catalytic property in early stage of diabetes.

REFERENCES

1) Olsen, W. A., and Rogers, L. (1972): Jejunal sucrase activity in diabetic rats. J. Lab. Clin. Med., 77, 838–842.
2) Younoszai, M. K., and Schedl, H. D. (1972): Effect of diabetes on intestinal disaccharidase activities. J. Lab. Clin. Med., 79, 579–586.
3) Caspary, W. F., Rhein, A. M., and Creutzfedt, W. (1972): Increase of intestinal brush border hydrolases in mucosa of streptozotocin-diabetic rats. Diabetologia, 8, 412–414.
4) Caspary, W. F. (1973): Effect of insulin and experimental diabetes mellitus on the digestive-absorptive function of the small intestine. Digestion, 9, 248–263.
5) Olsen, W. A., and Rosenberg, I. H. (1970): Intestinal transport of sugars and amino acids in diabetic rats. J. Clin. Invest., 49, 96–105.
6) Schedl, H. P., and Wilson, H. D. (1971): Effect of diabetes on intestinal growth and hexose transport in the rat. Am. J. Physiol., 220, 1739–1745.
7) Yamada, K., Goda, T., Sasaki, M., Moriuchi, S., and Hosoya, N. (1980): Effect of food restriction on intestinal disaccharidases in streptozotocin induced diabetes of rat. J. Nutr. Sci. Vitaminol., 26, 599–606.
8) Olsen, W. A., and Korosmo, H. (1975): Enhancement of intestinal sucrase activity in experimental diabetes: the role of intraluminal factors. J. Lab. Clin. Med., 85, 832–837.
9) Dahlqvist, A. (1964): Method for assay of intestinal disaccharidases. Anal. Biochem., 7, 18–25.
10) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265–275.
11) Yamada, K., Hosoya, N., Noda, S., and Moriuchi, S. (1980): Changes of immunoreactive sucrase-isomaltase complex in rat small intestine during postnatal development and maturation along the villus-crypt axis. J. Nutr. Sci. Vitaminol., 26, 171–182.
12) Mancini, G., Carbonara, A. O., and Heremans, G. F. (1965): Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2, 235–254.
13) Sasaki, M., Yamada, K., Moriuchi, S., and Hosoya, N. (1979): Purification and
characterization of rat intestinal sucrase-isomaltase complex. *Food Nutr.*, 32, 201–208.

14) Yamada, K., Moriuchi, S., and Hosoya, N. (1979): Some characteristics of early appearing isomaltase in intestinal mucosa of suckling rat. *J. Nutr. Sci. Vitaminol.*, 25, 545–552.

15) Nakabou, Y., Okita, C., Takano, Y., and Hagihira, H. (1974): Hyperplastic and hypertrophic changes of the small intestine in alloxan diabetic rats. *J. Nutr. Sci. Vitaminol.*, 20, 227–234.

16) Olsen, W. A., and Korsmo, H. (1977): The intestinal brush border membrane in diabetes. Studies of sucrase-isomaltase metabolism in rats with streptozotocin diabetes. *J. Clin. Invest.*, 60, 181–188.

17) Senegas-Balas, F., Balas, D., Bouisson, M., and Ribet, A. (1981): Effect of pancreatic duct ligation on the hamster intestinal mucosa. Variation of several hydrolases. *Digestion*, 21, 83–91.

18) Alpers, D. H., and Tedesco, F. J. (1975): The possible role of pancreatic proteases in the turnover of intestinal brush border proteins. *Biochim. Biophys. Acta*, 401, 28–40.

19) Sjöström, H., Norén, O., Christiansen, L., Waker, H., and Semenza, G. (1980): A fully active, two-active-site, single-chain sucrase–isomaltase from pig small intestine. *J. Biol. Chem.*, 255, 11332–11338.

20) Montgomery, R. K., and Sybicki, M. A. (1981): Rat intestinal microvillus membrane sucrase-isomaltase is a single high molecular weight protein and fully active enzyme in the absence of luminal factors. *Biochim. Biophys. Acta*, 661, 346–349.

21) Korc, M., Iwamoto, Y., Sankaran, H., Williams, J. A., and Goldfine, I. D. (1981): Insulin action in pancreatic acini from streptozotocin-treated rats. I. Stimulation of protein synthesis. *Am. J. Physiol.*, 240, G56–G62.

22) Nordström, C., Dahlqvist, A., and Josefsson, L. (1967): Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine. Comparison of alkaline phosphatase, disaccharidases, and dipeptidases. *J. Histochem. Cytochem.*, 15, 713–721.

23) Goda, T., Yamada, K., Sugiyama, M., Moriuchi, S., and Hosoya, N. (1982): Effect of sucrose and acarbose feeding on the development of streptozotocin-induced diabetes in the rat. *J. Nutr. Sci. Vitaminol.*, 28, 41–56.