Suppressive effect of insulin on the synthesis of sucrase–isomaltase complex in small intestinal epithelial cells, and abnormal increase in the complex under diabetic conditions

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An abnormally high level of the sucrase–isomaltase (SI) complex in the small intestine of rats with streptozotocin-induced insulin-dependent diabetes mellitus (IDDM) was normalized in 11 h by the administration of insulin, in addition to normalization of the blood glucose level. Phlorizin, an inhibitor of renal glucose re-absorption, also caused normalization of the blood glucose level in the IDDM rats; however, the level of the SI complex was barely changed. When mucosa explants were cultured in a medium, the SI complex synthesized during the cultivation was accumulated as its precursor protein without maturation, owing to the absence of pancreatic proteases, and the amount of the

precursor protein that accumulated in the explants was decreased by the addition of insulin into the medium. Further, the mRNA level of the SI complex in the explants incubated with insulin was obviously lower than that in the absence of insulin. These results indicate that insulin has a suppressive effect on the synthesis of the SI complex, presumably by decreasing the transcriptional level of the gene encoding the complex, in small-intestinal epithelial cells. Thus the synthesis of the SI complex might exceed normal levels in the epithelial cells as a direct result of the depletion of insulin under IDDM conditions.

INTRODUCTION

Sucrase and isomaltase form a complex enzyme (SI complex) on the brush border membranes of small-intestinal epithelial cells [1,2]. The SI complex is synthesized as a single precursor peptide, which is matured by cleavage into sucrase and isomaltase subunits by the action of pancreatic proteases on the brush border membranes. The intestinal epithelial cells are renewed continuously from the stem cells and are located near the lower region of the crypts, migrating from the crypts toward the top of the villus while maturing morphologically and biochemically [3,4]. The SI complex is not detectable in the immature epithelial cells in the crypts, but is induced during the maturation of the epithelial cells [5].

Diabetes mellitus is a state of nutrient starvation that frequently results in severe metabolic imbalances and pathological changes in many tissues. In the small intestine this severe disease causes significant changes in the morphology and function of the mucosa [6–8]. In diabetic patients and animals, in particular in rats with streptozotocin-induced insulin-dependent diabetes mellitus (IDDM), abnormal increases in the activities of sucrase and isomaltase are observed in the small intestine [9,10]. Furthermore it has been reported that the postprandial metabolic profile is improved by the administration of an inhibitor of the sucrase and isomaltase in the experimental IDDM rats [11], suggesting that the abnormal increases in these enzyme activities exert a harmful influence on the metabolic imbalance under diabetic conditions. However, mechanisms by which the sucrase and isomaltase activities in the small intestine are increased abnormally under IDDM conditions have not been clarified.

Here we report that the sucrase and isomaltase activities in the small intestines of the streptozotocin-induced IDDM rats become normal in a short time (11 h) after the administration of insulin, and that the synthesis of the SI complex is suppressed by the

direct action of insulin in the small-intestinal epithelial cells *in vitro*. On the basis of these findings we propose that the depletion of insulin directly induces abnormal synthesis of the SI complex in the epithelial cells under IDDM conditions.

MATERIALS AND METHODS

Chemicals

Regular and normal types of human recombinant insulin were kindly donated by Shionogi Pharmacy (Osaka, Japan). Bovine insulin was a product of Sigma. Anti-(rat SI complex) antiserum was raised in a rabbit immunized by injecting a purified preparation of the complex under the skin. The purified SI complex was prepared by the method of Takesu [12].

Animals

Male Wistar rats (6 weeks old), purchased from Kiwa Laboratory Animals Co. Ltd. (Wakayama, Japan) made diabetic by a single injection of streptozotocin (70 mg/kg body weight; dissolved in 100 mM citric acid buffer, pH 4.5) into the tail vein [13]. The rats were fed with a standard laboratory diet (CE-2; Clea, Osaka, Japan) from 17:00h to 20:00h *ad libitum* for 3 weeks, and then used for the experiments. At two weeks after the injection, induction of diabetes was confirmed by a determination of blood glucose level; animals showing a glucose level of 400 mg/dl or more were defined as diabetic rats.

Treatment with insulin and phlorizin

Immediately before feeding, the diabetic rats were injected with regular and normal types of human recombinant insulin (each 5

Abbreviations used: IDDM, insulin-dependent diabetes mellitus; SI complex, sucrase–isomaltase complex.

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i.u.) into the tail vein (the regular type) and under the skin (the normal type). Then the second (both types, each 4 i.u.) and third (the regular type alone, 1 i.u.) injections were made under the skin 3.5 and 5.5 h respectively after the first injection. For treatment with phlorizin, the diabetic rats were given the drug (450 mg/day per kg body weight; dissolved in propylene glycol) as a subcutaneous injection for 4 days.

Enzyme assay

At the time of killing, rats were anaesthetized with pentobarbital. The jejunum was divided into three segments of equal length; the middle segment was flushed with ice-cold PBS. The mucosa was scraped from this segment with a glass slide at 4 °C, then homogenized with 0.1 M potassium phosphate buffer, pH 6.8. After centrifugation at 3000 *g* for 10 min, the supernatant obtained was used as an enzyme solution. Sucrase and isomaltase activities were assayed with 25 mM sucrose and isomaltose respectively as substrate, by a published method [14]. One unit is defined as the amount of enzyme that hydrolyses 1 μ mol of sucrose or isomaltose in 1 min. DNA content in the mucosa was determined by the method of Croft and Lubran [15].

Explant culture

Small-intestinal explants were isolated from normal rats (6 weeks old) by a published method [16]. The explants were cultured in Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) fetal calf serum in the presence or absence of 100 nM bovine insulin.

Immunoblotting

The mucosa isolated from the middle segment of jejunum was homogenized in 0.1 M potassium phosphate buffer, pH 6.8, at 4 °C, and the supernatant obtained after centrifugation (3000 *g* for 10 min) was used as a sample solution for SDS/PAGE. The cultured explant was also homogenized in the same buffer for the preparation of a sample solution. The sample solution was subjected to SDS/PAGE [5 % or 7.5 % (w/v) gel] by the method of Laemmli [17], and the proteins in the gel were transferred to an Immobilon membrane (Millipore, Bedford, MA, U.S.A.). The membrane was incubated with a rabbit antiserum against rat SI complex, and immunoreactive proteins were detected with horseradish peroxidase-conjugated antibodies against rabbit IgG by using a commercial kit (Wako Pure Chemicals Industries, Osaka, Japan). Protein content in the sample solution was measured as described by Bradford [18].

Northern blotting

Total RNA (20 μ g) was extracted from the middle segment of jejunum or from cultured small-intestinal explants by a guanidinium isothiocyanate method [19], separated by electrophoresis through 1 % (w/v) agarose gel containing 6 % (v/v) formaldehyde, and transferred to a nylon membrane (Hybond-N+, Amersham). A 3 kb fragment of cDNA encoding rat SI complex [5] was kindly donated by Dr. Henning (Baylor College of Medicine, Houston, TX, U.S.A.), and digoxigenin-labelled cRNA was synthesized from the fragment by using a commercial kit (Boehringer Mannheim) and used as a probe. Hybridization was conducted with this probe (1 μ g/ml) in a 10 mM sodium phosphate buffer, pH 7.0, containing 50 % (v/v) formamide, 5 $\times$ Denhardt's solution [1 $\times$ Denhardt's being 0.1 % Ficoll/0.1 %

BSA/0.1 % poly(vinylpyrrolidone)], 2 $\times$ SSC (1 $\times$ SSC being 1.5 mM sodium citrate/15 mM NaCl), 0.1 % *N*-laurylsarcosine, 0.1 % SDS and 10 % dextran sulphate, at 42 °C for 16 h; the membrane was washed twice with 0.2 $\times$ SSC at 65 °C for 15 min and treated with 20 μ g/ml RNase in 10 mM Tris/HCl, pH 7.6, containing 1 mM EDTA and 500 mM NaCl, at 37 °C for 60 min. Control hybridization was conducted with a β -actin cRNA (400 bp) purchased from Boehringer Mannheim. Chemiluminescence signals derived from hybridized probes were detected on an X-ray film with a digoxigenin luminescence detection kit (Boehringer Mannheim), and quantified with a GS-250 Molecular Imager (Bio-Rad).

Statistical analysis

The results of the experiments are expressed as means $\pm$ S.E.M., and multiple comparisons of treatments were evaluated by means of Duncan's new multiple range test. Statistical significance was defined as $P < 0.05$.

RESULTS AND DISCUSSION

Effect of insulin administration on the levels of sucrase and isomaltase in streptozotocin-induced IDDM rats

To induce experimental IDDM, rats were given an intravenous injection of streptozotocin [13]. The IDDM rats were treated three times with insulin as follows: just before feeding, regular and normal types of human insulin (5 i.u. each) were injected into the tail vein (the regular type) and under the skin (the normal type), and then the second (both types, 4 i.u. each) and third (the regular type alone, 1 i.u.) injections were done under the skin 3.5 and 5.5 h respectively after the first injection. As shown in Figure 1, a prompt decrease in blood glucose level occurred after the insulin treatment, and the level reached nearly that of the normal rats 6 h after the first injection.

As reported previously [9,10], the specific activity of sucrase (units/mg of DNA) in the small intestine in the experimental

Figure 1 Normalization of the blood glucose level in the IDDM rats by the administration of insulin

The IDDM (○, ●) and normal (△) rats were treated with (○) or without (●, △) insulin as described in the Materials and methods section, and the blood glucose level was followed. Results are means $\pm$ S.E.M. for five rats. One typical result of four separate experiments (each $n = 4$ or 5) is shown.

Figure 2 Alteration of the sucrase activity in the small intestine after the administration of insulin in the IDDM rats

The IDDM (○, ●) and normal (△) rats were treated with (○) or without (●, △) insulin, and the sucrase activity in the small intestine was followed as described in the Materials and methods section. Results are means $\pm$ S.E.M. for five rats. * $P < 0.05$ compared with the normal rats (△); # $P < 0.05$ compared with the IDDM rats without the insulin treatment (●). One typical result of three separate experiments (each $n = 4$ or 5) is shown.

Figure 3 Immunoblot analysis of the sucrase and isomaltase subunits of the SI complex in the IDDM rats after the administration of insulin

The IDDM rats (b, c) and normal rats (a) were treated with (c) or without (a, b) insulin for 11 h, and the expression levels of the sucrase and isomaltase subunits of the SI complex in the small intestine were determined by SDS/PAGE [7.5% (w/v) gel] followed by immunoblotting with an antiserum against rat SI complex as described in the Materials and methods section (10 μ g of protein was subjected to SDS/PAGE). One typical result of three separate experiments (each $n = 3$ or 4) is presented.

IDDM rats was more than twice that in the normal rats (Figure 2). After the IDDM rats had been treated with insulin, the sucrase activity was normalized in 11 h and continued at a normal level for at least an additional 13 h. Similar results were obtained when isomaltase activity was examined (results not shown). The normalization of the sucrase and isomaltase activities was confirmed when the expression levels of the sucrase and isomaltase subunits of the SI complex were examined by SDS/PAGE followed by immunoblotting with an antiserum against rat SI complex (Figure 3). Moreover, Northern blot analysis showed that the mRNA level of the SI complex in the IDDM rats, which was about twice that in the normal rats, was decreased to the normal level in 11 h by the insulin treatment

Table 1 Effect of insulin administration on the mRNA levels of the SI complex in IDDM rats

The IDDM rats were treated with or without insulin for 11 h as described in the Materials and methods section, and the mRNA level of the SI complex in the small intestine was determined by Northern blot analysis. Results are means $\pm$ S.E.M. ($n = 5$) normalized with β -actin mRNA level, and are presented as relative values (percentage of those in the normal rats). * $P < 0.05$ compared with the normal rats; † $P < 0.05$ compared with the IDDM rats not treated with insulin.

Rats	mRNA level of the SI complex (% of normal)
Normal	100 $\pm$ 12.8
IDDM, without insulin	213 $\pm$ 9.1*
IDDM, with insulin	119 $\pm$ 18.8†

Table 2 Effect of phlorizin treatment on sucrase activity in the small intestine of IDDM rats

The IDDM rats were treated with or without phlorizin for 4 days as described in the Materials and methods section, and the blood glucose level and sucrase activity in the small intestine were determined. Results are means $\pm$ S.E.M. for five rats. * $P < 0.05$ compared with the normal rats; † $P < 0.05$ compared with the IDDM rats not treated with phlorizin. One typical result of three separate experiments (each $n = 4$ or 5) is shown.

Rats	Blood glucose level (mg/dl)	Sucrase activity (units/mg of DNA)
Normal	126 $\pm$ 4	2.6 $\pm$ 0.53
IDDM, without phlorrhizin	506 $\pm$ 143*	9.8 $\pm$ 0.81*
IDDM, with phlorrhizin	102 $\pm$ 49†	8.9 $\pm$ 1.30*

(Table 1). These results indicate that in the IDDM rats the treatment with insulin normalizes the expression level of the SI complex, presumably by decreasing the transcriptional level of the gene encoding the complex.

Relationship between the level of the SI complex in the small intestine and the blood glucose level in the IDDM rats

Many kinds of abnormal glucose metabolism have been reported under diabetic conditions, and it is recognized that in many cases hyperglycaemia itself might have a pathogenic role in diabetes mellitus [20–22]. Takeguti et al. [23] observed that glucose infusion into normal rats induced an increase in the sucrase activity in the small intestine, corresponding to an increase in the blood glucose level. Furthermore it was reported that glucose absorbed from the lumen through the sodium–glucose co-transporter up-regulated the expression level of the SI complex in the small intestine [24]. It is thus suspected that the abnormally high expression level of the SI complex under IDDM conditions is a result of hyperglycaemia. To elucidate the relationship between the expression level of the SI complex and the blood glucose level, the sucrase activity in the small intestine was examined after the administration of phlorizin to the IDDM rats. Phlorizin, which is an inhibitor of renal glucose re-absorption, has been reported to lead to a nearly normal blood glucose level without the participation of insulin in IDDM rats [20]. As shown in Table 2, the sucrase activity was barely changed after the administration of phlorizin, although the blood glucose level was normalized. These results clearly indicate that normalization of the blood glucose level without the participation of insulin does not result in a decrease in the level of the SI complex in the small intestine of IDDM rats.

Figure 4 Effect of insulin on the synthesis of the precursor protein of the SI complex in cultured small-intestinal explants

Small-intestinal explants isolated from a normal rat were preincubated in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum for 1 h, then treated with (lane 2) or without (lane 3) bovine insulin (100 nM) for 0 h (lane 1) or 10 h (lanes 2 and 3). The precursor protein of the SI complex synthesized during the incubation was analysed by SDS/PAGE [5% (w/v) gel] followed by immunoblotting (40 µg of protein was subjected to SDS/PAGE). Details are given in the Materials and methods section. One typical result of six separate experiments with different rats is presented.

Figure 5 mRNA level of the SI complex in cultured small-intestinal explants treated with insulin

Small-intestinal explants isolated from a normal rat were preincubated in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum for 1 h, then treated with (lane 3) or without (lane 2) bovine insulin (100 nM) for 0 h (lane 1) or 5 h (lanes 2 and 3). The mRNA levels of the SI complex (upper panel) and β -actin (lower panel) were examined by Northern blot analysis. Details are given in the Materials and methods section. One typical result of five separate experiments with different rats is presented.

Down-regulation of the SI complex by insulin in small-intestinal explants

To elucidate the direct effect of insulin on the expression level of the SI complex in the brush border membranes, mucosa explants were excised from the jejunum of normal rats and incubated in a medium supplemented with or without insulin. The precursor protein of the SI complex, which has a molecular mass of 260 kDa [16], was found in the explants after incubation for 10 h in the absence of insulin (Figure 4, lane 3). This result is consistent with a previous report showing that newly synthesized SI complex is accumulated as the precursor protein without maturation in cultured mucosa explants because of the absence of pancreatic proteases that cleave the precursor protein into the sucrase and isomaltase subunits of the mature protein [16].

However, when insulin was added to the medium, the amount of the precursor protein accumulated during the incubation was decreased significantly (Figure 4, lane 2), indicating that the synthesis of the SI complex is suppressed by insulin in the explants. Similar results were obtained when mucosa explants derived from the streptozotocin-induced IDDM rats were used (results not shown). When the mRNA level of the SI complex in the mucosa explants from the normal rats was examined by Northern blot analysis, as shown in Figure 5, the mRNA level in the explants incubated with insulin was obviously low (lane 3), compared with that in the absence of insulin (lane 2).

Our present results indicate that insulin directly down-regulates the synthesis of the SI complex, presumably by decreasing the transcriptional level of the gene encoding the complex, in the small-intestinal epithelial cells. It has been reported that the expression level of the insulin receptor increases during differentiation of the epithelial cells of the small intestine [25]. Furthermore the insulin receptor has been reported to be expressed in the cells even under IDDM conditions [26]. It is therefore reasonable to postulate that under IDDM conditions synthesis of the SI complex is exceedingly high in the epithelial cells, owing to the depletion of insulin, resulting in the abnormal increases in the sucrase and isomaltase activities in the small intestine.

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