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Insulin deficiency induces abnormal increase in intestinal disaccharidase activities and expression under diabetic states, evidences from in vivo and in vitro study

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ABSTRACT

Structural and functional alterations in the gastrointestinal tract of diabetic patients are often accompanied by increase in absorption of intestinal glucose and activities of brush-border disaccharidases. The purpose of this study was to investigate the role of insulin in regulating intestinal disaccharidases using in vivo and in vitro experiments. Streptozotocin-induced diabetic rats and normal rats received protamine zinc insulin (10 IU/kg) subcutaneously twice daily for 5 weeks. Disaccharidase activities and sucrase–isomaltase (SI) complex protein and mRNA expression in intestinal regions were assessed. In addition, Caco-2 cells were cultured in medium containing glucose, insulin or insulin plus some pharmacological inhibitors for 7 days, disaccharidase activities, sucrase–isomaltase (SI) complex and Cdx2 mRNA levels were measured. The animal experiments showed that diabetes increased intestinal disaccharidase activities, accompanied by high mRNA and protein expression of SI complex. Insulin treatment reversed the increases induced by diabetes. The cellular results showed that insulin suppressed disaccharidase activities and down-regulated SI complex and Cdx2 mRNA expression in a concentration-dependent manner. The inhibitor of MAPK signal pathway PD-98059 blocked the suppression of disaccharidase activities and expression of SI complex and Cdx2 mRNA induced by insulin. In conclusion, insulin deficiency induces abnormal increase in intestinal disaccharidase activities and expression under diabetic states. Insulin plays an essential role in regulation disaccharidase activities and expression, at least in part, via the MAPK-dependent pathway.

1. Introduction

Carbohydrates are necessary for energy and ultimately digested by α-glucosidase and disaccharidases in the small intestine. Carbohydrate digestion directly induces the increase of postprandial blood glucose levels [1]. Sucrase and isomaltase, which form a complex enzyme (sucrase–isomaltase complex, SI complex) on the brush border membranes [2], are two major components of disaccharidases and play important roles in the final carbohydrate digestion. The SI complex is synthesized as a single precursor peptide, which is matured by cleavage into sucrase and isomaltase subunits via pancreatic proteases [3].

Diabetes mellitus is associated with the postprandial hyperglycemia which is considered to be a high-risk factor resulting in the development of chronic complications of diabetes mellitus [4]. A series of reports have been showed that activities of disaccharidases including sucrase and isomaltase are abnormally high in small intestine of diabetic patients [5] and experimental diabetic animals [6–8], which indicates that the increase in the activities of disaccharidase is one of the factors resulting in postprandial hyperglycemia in diabetic states.

Insulin deficiency is a significant feature of diabetes mellitus. Our previous studies showed that the increased activities of intestinal disaccharidases in streptozotocin-induced diabetic rats was accompanied by low insulin levels [6,9], and increased activities of disaccharidases were reversed by administration of insulin [9]. This suggests that insulin may be a potential factor regulating the activities of intestinal disaccharidases in the diabetic rats.

The Caco-2 cells, derived from human colon adenocarcinoma, are described to display some intestinal epithelial characteristics

Abbreviations: SI, sucrase–isomaltase; STZ, streptozotocin; FBG, fasted blood glucose; PKC, protein kinase C; PKA, protein kinase A; PI3K, phosphoinositide 3-kinase; MAPK, mitogen-activated protein kinase; Cdx2, caudal type homeobox 2; I-OMe-AG538, α-cyano-(3-methoxy-4-hydroxy-5-iodocinnamoyl)-(3′,4′-dihydroxyphenyl) ketone, H-89, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride; LY294002, 2-(4-morpholinyl)-8-phenyl-1(4H)-benzopyran-4-one hydrochloride; PD-98059, 2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one; ANOVA, analysis of variance.

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including a small-bowel phenotype with microvilli, dome formation, and the expression of SI complex [10–12]. The cell model is widely used as a human model to study the disaccharidase activities [6,13,14].

The aim of this study was to investigate: (1) effect of insulin treatment on activities and expressions of intestinal disaccharidases in both STZ-induced diabetic rats and normal rats; (2) insulin-mediated activities of intestinal disaccharidases in Caco-2 cells; and (3) some intracellular signaling pathways involved in regulation of intestinal disaccharidase activities induced by insulin using Caco-2 cells. It is expected to obtain an understanding of the mechanism regulating activities of intestinal disaccharidases by insulin.

2. Materials and methods

2.1. Experimental animals

Male Sprague-Dawley rats, weighing 180–200 g, were supplied by Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). The rats were maintained in an air conditioned animal quarter at a temperature of 22 ± 2 °C and a relative humidity of 50 ± 10% with a 12 h light/12 h darkness cycle. Water and food (laboratory rodent chow, Nanjing, China) were allowed ad libitum. The animals were acclimatized to the facilities for 5 days and fasted with free access to water for 8 h prior to experiment. The studies were approved by the Animal Ethics Committee of China Pharmaceutical University, and every effort was made to minimize stress to the animals.

2.2. Diabetic rats induced by streptozotocin

The diabetic rats were induced by an intraperitoneal administration of 55 mg/kg of streptozotocin (STZ, Sigma Chemical Co., St. Louis, MO, USA), dissolved in sodium citrate buffer, pH 4.5. Age-matched normal rats were injected with the vehicle (sodium citrate buffer, pH 4.5). On day 7 post-STZ injection, the fasting blood glucose levels were measured using commercially available glucose kit (Jian-cheng Biotech Co., Nanjing, China) based on glucose oxidase method. Rats with fasting blood glucose levels in excess of 11.1 mM were considered to be diabetic rats [15].

2.3. Drug treatment

On the seventh day post-STZ injection, the diabetic rats were randomly divided into two groups. Group 1 was served as diabetic control and only received vehicle. Group 2 was treated with 10 IU/kg of protamine zinc insulin (Wanbang Biopharmaceutical Co., Xuzhou, China) subcutaneously twice daily for 35 days. Effect of insulin treatment on normal rats was also studied. The fasting blood glucose, food uptake, and body weight were monitored on day 7, 14, 21, 28, and 35 of drug treatment.

On day 36 of the treatment, the rats fasted for 8 h were sacrificed under ether anesthesia; three regions (duodenum, jejunum, and ileum) of intestine were immediately removed, gently flushed with ice-cold physiological saline. A part of intestinal regions (2 cm length) were used to prepare crude enzyme solution for disaccharidase activity and western blot analysis. The rested intestinal regions were frozen in liquid nitrogen for QT-PCR analysis.

2.4. Measurement of disaccharidase activities in small intestine

The crude enzyme solution from small intestine was prepared according to procedure previously described [6,16]. The mucosa of individual intestinal region (about 2 cm length) was collected by scraping with a glass slide and homogenized in 1 ml of 100 mM ice-cold potassium phosphate buffer, pH 6.8. After centrifugation at 3000 × *g* for 10 min, the supernatant was used as crude enzyme solution. The activities of intestinal disaccharidase were measured by determination of glucose released from maltose and sucrose according to method described previously [9,17]. Briefly, the homogenate supernatants were diluted and added to an equal volume of 0.1 M sodium maleate buffer (pH 6.0) containing 56 mM sucrose or maltose, and were incubated for 1 h at 37 °C. The mixtures were then added to the glucose oxidase–peroxidase reagents (Jian-cheng Biotech Co., Nanjing, China) containing o-dianisidine as a chromogen, and the absorbance was measured at 420 nm. Protein content was measured using Bradford dye assay, using bovine serum albumin as a standard. The disaccharidase activities are expressed as U/mg protein. One unit is defined as the amount of enzyme that hydrolyses 1 mmol of sucrose or maltose in 1 min.

2.5. QT-PCR analysis

QT-PCR analysis was used to measure SI mRNA levels in rat duodenum and jejunum as well as Caco-2 cell. Briefly, total RNA was extracted from frozen tissue or cells using Trizol reagent (Invitrogen Co., Carlsbad, CA, USA) following the standard RNA isolation method. The quality of RNA was checked using the DU640 nucleic acid analyzer (Beckman Co., Gaithersburg, MD, USA). Two milligrams of total RNA from each original sample was converted into cDNA for each individual QT-PCR assay in a 40-cycle three-step PCR using the ABI Prism 7000 thermocycler. PCR primer sequences (Yingjun Biotech, Shanghai, China) are shown in Table 1. Amplification was performed in 20 μL reaction mixture: 2.0 μL of 10× PCR buffer, 2.0 μL of 25 mM $MgCl_2$, 0.4 μL of 10 mM deoxyribonucleoside triphosphate, 250 nM of the appropriate forward and reverse primers (SI, CDX2 and β-actin), and SYBR green I (Invitrogen Co., Carlsbad, CA, USA). For normalization of the gene levels, GAPDH was used to correct minor variations in the input RNA amount or inefficiencies of the reverse transcription. The results were calculated according to Applied-Biosystems.

2.6. Western blot analysis

The crude enzyme solution (10 μg of protein) was subjected to SDS-PAGE (7.5% gel) according to Yasuda [18]. Proteins in the gel were transferred to a polyvinylidene difluoride membrane (Millipore Co., Billerica, MA, USA). The membrane was blocked in phosphate-buffered saline containing 0.1% Tween-20 (PBST) and 5% dried skim milk for 60 min at room temperature and washed

Table 1
Primer characteristics of SI, CDX2 and GAPDH mRNA.

Gene	Accession no.	Forward primer	Reverse primer
SI (rat)	XM_346624	5′-GGAGGTTACATTCTACCATGTCAAG-3′	5′-CCAGGTGATTTGTATTGGTTCATCA-3′
GAPDH (rat)	XM_216453	5′-GGTGCTGAGTATGTCGTGGAG-3′	5′-ATGCAGGGATGATGTTCTGG-3′
SI (human)	NM_001041	5′-ATCATCCCTACCCAGGAACC-3′	5-GCTGGTCATTTTCACCCACT-3′
Cdx2 (human)	NM_001265	5′-GAGCACGGACACTGTGAGAA-3′	5′-GAAAGCTTGGTGCCTGTAGC-3′
GAPDH (human)	NM_002046	5′-TGACGGGGTCACCCACACTGTGCCCATCTA-3′	5′-CTAGAAGCATTTGCGGTGGACGATGGAGGG-3′

three times for 15 min in PBST. Then the membrane was incubated with the SI polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA. USA), diluted 500-fold in PBST overnight at 4 °C. After removal of the primary antibody, the membrane was washed with PBST and then it was incubated in the appropriate HRP-conjugated goat anti-mouse secondary antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at room temperature for another 1 h and washed again three times in PBST. The transferred proteins were incubated with enhanced chemiluminescence substrate solution for 5 min and visualized with autoradiography X-film. The relative levels were quantified densitometrically by using the quantity one software (Bio-Rad Laboratories, Richmond, CA, USA) and calculated according to the reference bands of glyceraldehydes phosphate dehydrogenase (GAPDH).

2.7. Cell culture

The Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD, USA). The Caco-2 cells (passages 19–32) were grown in DMEM (Invitrogen Co., Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen Co., Carlsbad, CA, USA), 1% nonessential amino acids (Invitrogen Co., Carlsbad, CA, USA), 4 mmol/L of glutamine, 1×10^5 U/L of penicillin, and 100 mg/L of streptomycin according to previous studies [6,19].

2.8. Effect of glucose and insulin on disaccharidase activities in Caco-2 cells

Cells were seeded at a density of 1×10^5 cells per well in six-well culture plates (Corning Co., Lowell, MA, USA). Cells were cultured for 2 days in the free-drug medium, then cultured for another 7 days in medium containing different concentrations of glucose (2, 10, 25 and 35 mM) or insulin (0.1, 0.5, and 2.5 IU/L). The cells were collected for disaccharidase assay and QT-PCR analysis. 3-(4,5-Dimethyl-2-thiazoyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Invitrogen Co., Carlsbad, CA, USA) assay showed that the tested agents used in the study had no damage on the viability of cells within the tested concentrations.

The activities of sucrase and maltase were measured using our method previously reported [6,20]. In brief, cell monolayers were washed with ice-cold phosphate-buffered saline and then collected. The collected cells underwent ultrasonic disruption in 0.1 ml glucose-free Hanks' balanced salt solution, and 0.1 mL of maltose or sucrose solution (56 mM) was added. The mixture was incubated at 37 °C for 60 min. The reaction was stopped by adding 0.1 mL of methanol. The amount of glucose released from the substrates was measured using HPLC–UV method previously reported [21]. The amount of glucose (micromole) produced in 1 h is defined as unit of disaccharidase activities.

2.9. Effect of pharmacological inhibitors on disaccharidase activities mediated by insulin in Caco-2 cells

Cells were seeded at a density of 1×10^5 cells per well in six-well culture plates. Cells were cultured for 2 days in the free-drug medium, then were cultured for another 7 days in the medium containing insulin (2.5 IU/L) co-administrated with insulin receptor polyclonal antibody (0.2 μg/mL, Millipore Co., Billerica, MA, USA), I-OMe-AG538 (10 μM), H-89 (10 μM), LY294002 (10 μM), chelerythrine (10 μM), or PD-98059 (10 μM). These chemical inhibitors were purchased from Sigma–Aldrich Shanghai Trading Co. (Shanghai, China) and the concentrations of the pharmacological inhibitors were selected according to several Refs. [6,22–24]. The cells were collected for disaccharidase assay and QT-PCR analysis.

2.10. Data analysis

Results are expressed as mean ± standard deviation (SD). Statistical differences among groups were evaluated by one-way analysis of variance. If analysis was significant, the differences between groups were estimated using Student–Newman–Keuls multiple comparison post hoc test. A p value of less than 0.05 indicated a significant difference.

3. Result

3.1. Effects of insulin treatment on disaccharidase activities in intestine of diabetic rats

As expected, a single administration of STZ to rats produced diabetic symptoms including loss of body weight, significant increases in both blood glucose level ($p < 0.01$) and food uptake ($p < 0.01$). Insulin treatment significantly decreased the fasting blood glucose levels, accompanied by the improvement of body weight and reduction of food intake (Fig. 1). Both sucrase and maltase were selected for evaluating disaccharidase activities. It was found that activities of sucrase and maltase in the interested intestinal regions (duodenum, jejunum, and ileum) of diabetic rats were significantly higher than those in normal control rats (Fig. 2). Compared with normal control rats, diabetes resulted in 2.52-, 2.65-, and 3.14-fold increase in sucrase activity and 2.21-, 2.58-, and 4.34-fold increase in maltase activity of duodenum, jejunum, and ileum, respectively. Insulin treatment almost restored disaccharidase activities in intestinal regions of diabetic rats to the level of normal control rats. All these results demonstrated that diabetes induced significant increase in intestinal disaccharidase activities, and insulin treatment reversed the increase in disaccharidase activities under diabetic states. It was contrast to results found in diabetic rats that insulin treatment showed a trend to increase activities of both sucrase and maltase in small intestine of normal rats. The increase was associated with lower blood glucose level in normal rats treated with insulin.

3.2. Effects of insulin treatment on levels of SI complex protein and mRNA in intestine of diabetic rats

Sucrase and isomaltase are originally expressed as SI complex. The expression of SI complex in jejunum and ileum of experimental rats were measured using western bolting. The results showed that diabetes significantly increased expression of SI complex protein in the jejunum and ileum of rats, inducing 5.23- and 6.36-fold increase of normal rats in jejunum and ileum, respectively. Insulin treatment reversed the increased SI complex protein level induced by diabetes.

Data from QT-PCR analysis showed that similarly to expression of SI complex protein, diabetes also increased levels of SI complex mRNA in the duodenum and jejunum of diabetic rats, which were 8.14- and 9.45-fold of that in the normal control rats, respectively (Fig. 3). Insulin treatment almost restored levels of SI complex mRNA expression in diabetic states to those in normal rats. It was also observed that insulin treatment raised levels of SI complex protein and mRNA in the indicated intestinal regions of normal rats, which was in an agreement with the findings that insulin treatment increased disaccharidase activities in intestine of normal rats. The alterations in levels of both SI complex protein and mRNA were in parallel with results in disaccharidase activities.

3.3. Effects of glucose and insulin on disaccharidase activities in Caco-2 cells

Diabetes mellitus is characterized with the hyperglycemia and insulin deficiency, the effect of both glucose and insulin on

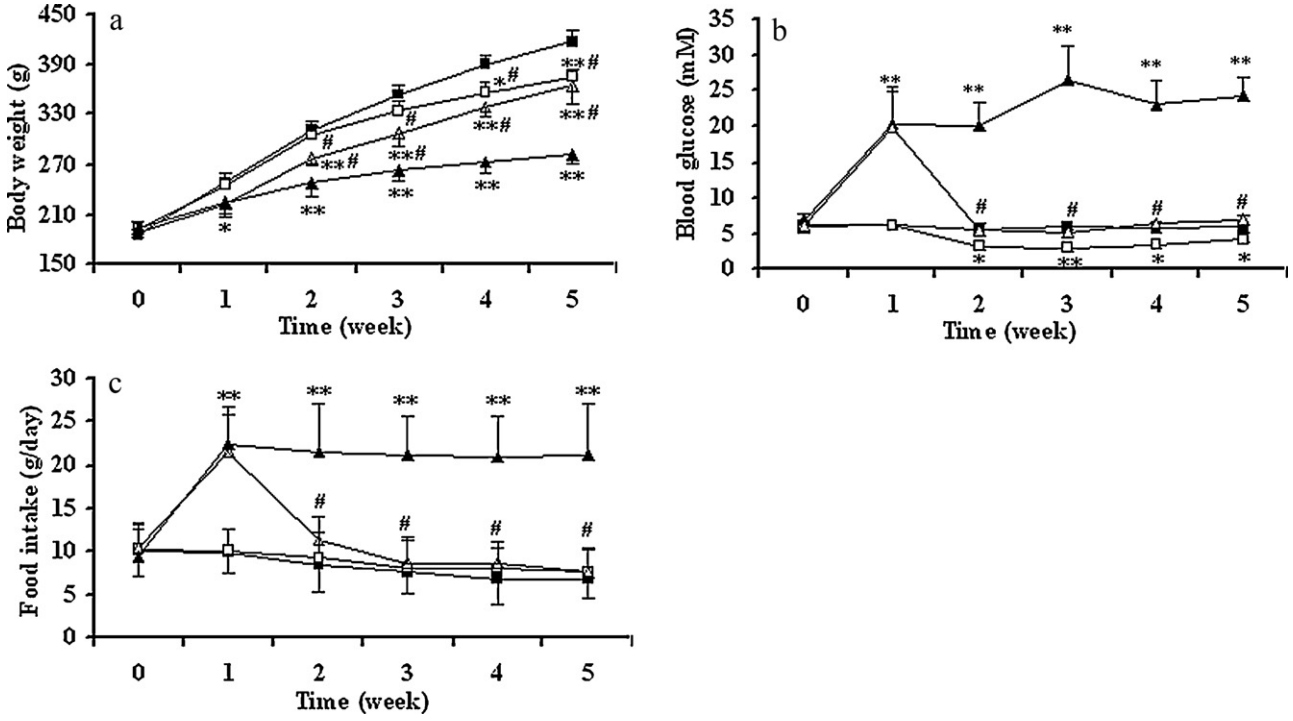

Fig. 1. Effects of insulin on body weight (a), fasting blood glucose level (b), food intake (c) during the 5-week treatment in normal and STZ-induced diabetic rats. Symbols represent as follows: *filled square*, normal control rats; *open square*, normal rats treated with 10 IU/kg of insulin; *filled triangle*, diabetic control rats; *open triangle*, diabetic rats treated with 10 IU/kg of insulin. The results are expressed as mean ± SD ($n = 6$), $^{*}p < 0.05$, $^{**}p < 0.01$ vs. normal control rats; $^{\#}p < 0.01$ vs. diabetic control rats.

disaccharidase activities in Caco-2 was investigated. The cells were cultured in medium containing different concentrations of glucose (2, 10, 25 and 35 μM) or insulin (0.1, 0.5, and 2.5 IU/L) for 7 days and the disaccharidase activities were measured (Fig. 4). It was found that glucose inhibited sucrase and maltase activities in a concentration-dependent manner. Two micromoles of glucose induced 2.24- fold and 2.45-fold increase of sucrase and maltase in the cells cultured in 25 μM of glucose (control).

Effect of insulin on disaccharidase activities in Caco-2 cells was further studied. The results showed that that insulin also inhibited sucrase and maltase activities in a concentration-dependent manner. Data from QT-PCR analysis demonstrated that insulin attenuated SI complex mRNA expression in Caco-2 cells. Significant reduction in expression of SI complex mRNA was showed in cells cultured with both 0.5 and 2.5 IU/L of insulin (Fig. 5).

The homeobox gene Cdx2 is consider to be a key transcriptional factor which involved in the regulation of the expression of intestine specific markers such as SI gene. The level of Cdx2 mRNA was also measured in the study (Fig. 5). Similar reduction was found in cells cultured with insulin.

3.4. Effects of pharmacological inhibitors on disaccharidase activities mediated by insulin in Caco-2 cells

The present studies clearly demonstrated that insulin suppressed disaccharidase activities and SI complex mRNA expression in a concentration-dependent manner. Effects of some pharmacological inhibitors on disaccharidase activities and SI complex mRNA expression mediated by insulin were documented. Caco-2 cells were cultured in medium containing insulin co-administrated with different inhibitors for 7 days. Data for disaccharidase activities (Fig. 6a and b) showed that insulin receptor antibody, I-OMe-AG538 (a tyrosine protein kinase inhibitor), and PD-98059 (a MAPK inhibitor) reversed the decrease in disaccharidase activities induced by insulin, but H-89 (a PKA inhibitor), LY294002 (a PI3K/Ait inhibitor) and chelerythrine (a PKC inhibitor) did not affect alterations in disaccharidase activities induced by insulin. These pharmacological inhibitors themselves showed no inhibitory effects on disaccharidase activities (Fig. 6c and d). Further investigations showed that that insulin receptor antibody, I-OMe-AG538 and PD-98059 reversed the down-regulation of SI

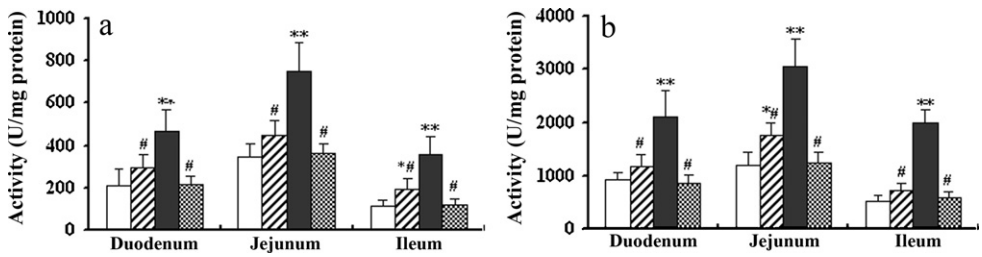

Fig. 2. Effects of 5-week treatment with insulin on sucrase (a) and maltase (b) activity in the small intestine of normal and STZ-induced diabetic rats. Symbols represent as follows: *white bar*, normal control rats; *shaded bar*, diabetic control rats; *diagonally striped bar*, normal rats treated with 10 IU/kg of insulin and *hatched bar*, diabetic rats treated with 10 IU/kg of insulin. The results are expressed as mean ± SD ($n = 6$), $^{*}p < 0.05$, $^{**}p < 0.01$ vs. normal control rats; $^{\#}p < 0.01$ vs. diabetic control rats.

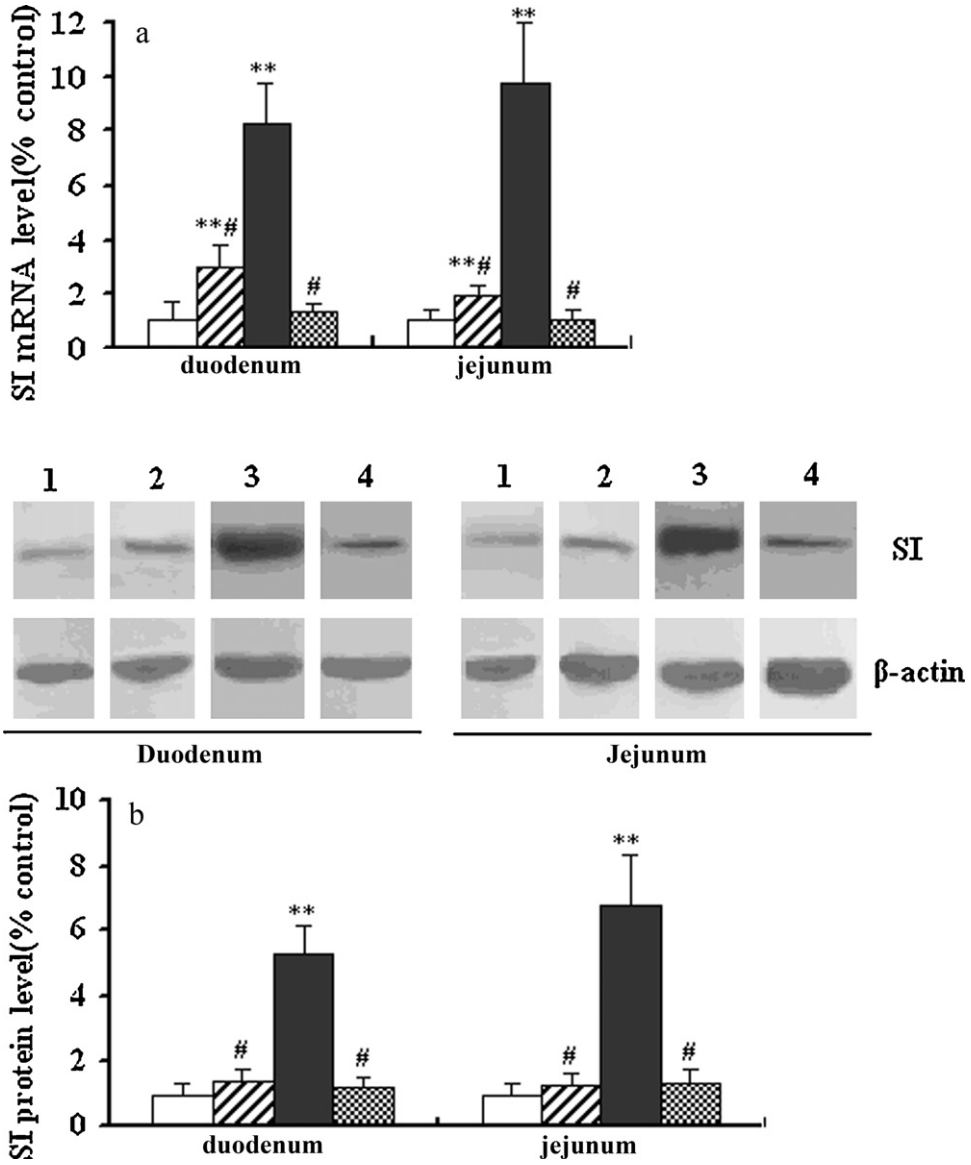

Fig. 3. Effects of 5-week treatment with insulin on SI complex mRNA (a) and protein (b) expression in the small intestine of normal and STZ-induced diabetic rats. *white bar*, normal control rats; *shaded bar*, diabetic control rats; *diagonally striped bar*, normal rats treated with 10 IU/kg of insulin and *hatched bar*, diabetic rats treated with 10 IU/kg of insulin. The results are expressed as mean ± SD ($n = 4$), $^{**}p < 0.01$ vs. normal control rats; $^{\#}p < 0.01$ vs. diabetic control rats.

complex mRNA expression and Cdx2 mRNA induced by insulin (Fig. 5).

4. Discussion

Carbohydrates are digested into glucose by α-glucosidase and disaccharidases in the small intestine, which directly induces the increase in postprandial blood glucose levels. The significant increase in disaccharidase activities is observed in experimental animals [6,9,25,26] and diabetic patients [5,27], which becomes an important factor leading to the elevated blood glucose levels. SI complex is a brush border enzyme with an important function in degradation of disaccharides [28]. Its expression is characterized by a strong expression at the crypt–villus junction and mid-villus, and a decreased intensity towards the tips to the villi. SI gene is specifically expressed by enterocytes in a differentiation-specific pattern and a widely used marker for intestinal differentiation [12]. Our results showed the increase in disaccharidase activities and high expression of SI complex protein in intestine of diabetic rats, which may result from abnormal intestinal hyperplasia and hypertrophy [29–32]. Insulin deficiency is a significant feature of diabetes, which indicated that low level of insulin may be a main reason resulting in the increased disaccharidase activities under diabetic state. This hypothesis was supported by our findings that the diabetes increased disaccharidase activities and insulin treatment reversed the alterations induced by diabetes. Similar results were found in other reports [27,33]. Sucrase and isomaltase were originally expressed as SI complex. The protein and mRNA levels of SI complex were further investigated. The results clearly demonstrated that protein and mRNA levels of SI complex were markedly elevated and insulin treatment restored the increased values to those of normal rats. The alteration in protein and mRNA levels of SI complex was in line with the alteration in disaccharidase activities, which indicated that insulin directly down-regulates the synthesis of the SI complex, presumably by decreasing the transcriptional level of the gene encoding of the complex, in the small-intestinal epithelial cells. However, it was contrast to the findings in diabetic rats that insulin treatment increased disaccharidase activities, protein and mRNA levels of SI complex in normal rats, accompanied by low level of blood glucose.

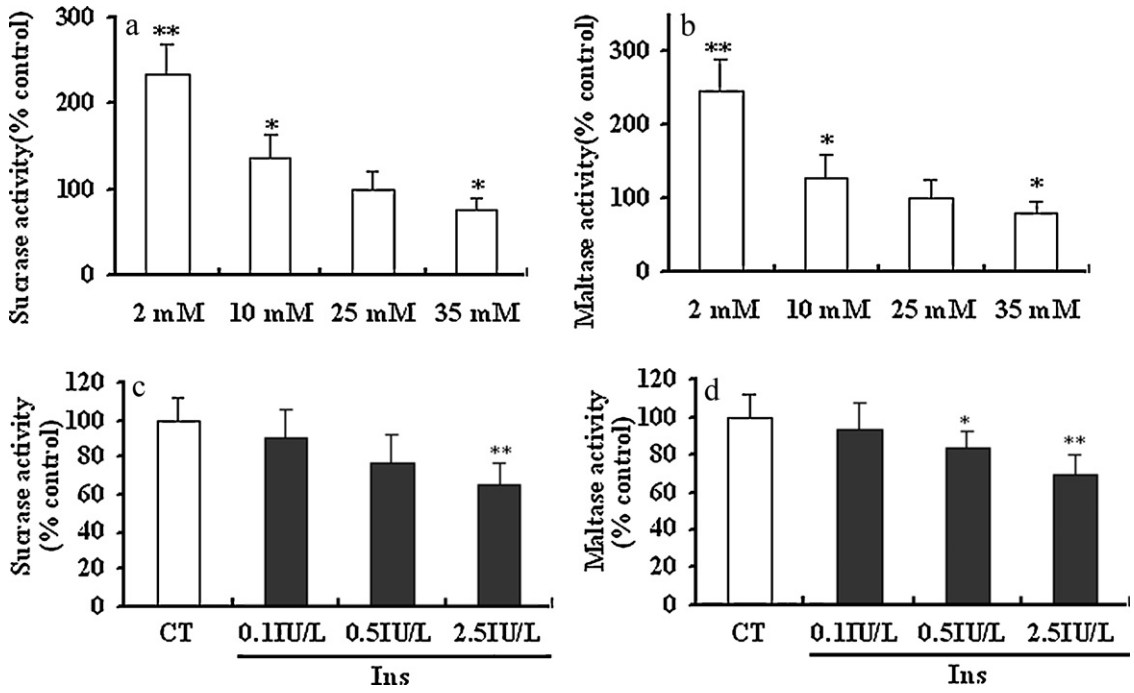

Fig. 4. Effects of glucose on sucrase (a) and maltase (b) activity in Caco-2 cells. Effects of insulin (Ins) on sucrase (c) and maltase (d) activity in Caco-2 cells. The Caco-2 cells were cultured in medium containing insulin or glucose for 7 days. The control cells were cultured with 25 mM of glucose. The results are expressed as mean ± SD ($n = 6$), $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control cells (CT).

The low level of blood glucose may account for the increases in disaccharidase activities, protein and mRNA levels of SI complex in insulin-treated normal rats.

The Caco-2 cells, which has been used as an in vitro model for the studies of disaccharidase activities [6,19,34], was also introduced into the present study. Hyperglycemia is a bright character under diabetic states, data from disaccharidase activities showed that disaccharidase activities were negatively regulated by glucose. This finding was in agreement with negative regulation of SI complex induced by glucose [35]. The results excluded the contribution of hyperglycemia to the increase in disaccharidase activities under diabetic state and may partly explain the in vivo finding that insulin treatment increased disaccharidase activities, protein and mRNA levels of SI complex in normal rats.

Further experiment was focused on roles of insulin in regulating disaccharidase activities. As we expected, insulin suppressed both disaccharidase activities and protein and mRNA expression of SI complex. Besides that, the suppression could be reversed by adding insulin receptor polyclonal antibody. Good association between low disaccharidase activities and low mRNA levels of SI complex were found in cells treated with insulin, indicating that the suppression of mRNA expression of SI complex may account for the decrease in disaccharidase activities. It is well-known that insulin shows its biological activities via PKC, PKA, PI3K/Akt or MAPK signal pathways. Accumulating evidences have also showed that PKC [36], PKA [23,37], PI3K/Akt [38,39] and MAPK [24,40] are involved in proliferation, differentiation as well as SI complex expression in intestinal epithelial cells, which indicated that the inhibitory effects of insulin on disaccharidase activities, and mRNA expression of SI complex were involved in these signal pathways. To support the above hypothesis, disaccharidase activities were measured in Caco-2 cells cultured in medium containing insulin and specific pharmacological inhibitors including I-OMe-AG538 (a tyrosine protein kinase inhibitor), PD-98059 (a MAPK inhibitor), H-89 (a PKA inhibitor), LY294002 (a PI3K/Akt inhibitor) and chelerythrine (a PKC inhibitor). Only I-OMe-AG538 and PD-98059 were found to reverse the decrease in disaccharidase activities and mRNA expression of SI complex induced by insulin.

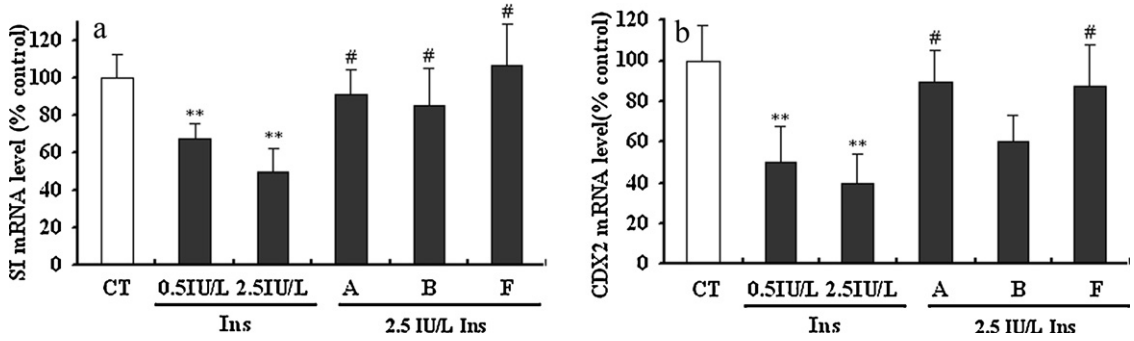

Fig. 5. The expression of SI mRNA level (a) and CDX2 mRNA level (b) when Caco-2 cells were cultured in medium containing insulin (Ins) and pharmacological inhibitors including insulin receptor antibody (A, 0.2 μg/mL), tyrosine kinase inhibitor I-OMe-AG538 (B, 10 μM) and MAPK inhibitor PD-98059 (F, 10 μM) for 7 days. The results are expressed as mean ± SD ($n = 4$), $^{**}p < 0.01$ vs. control cells (CT); $^{\#}p < 0.01$ vs. cells treated with Ins (2.5 IU/L) alone.

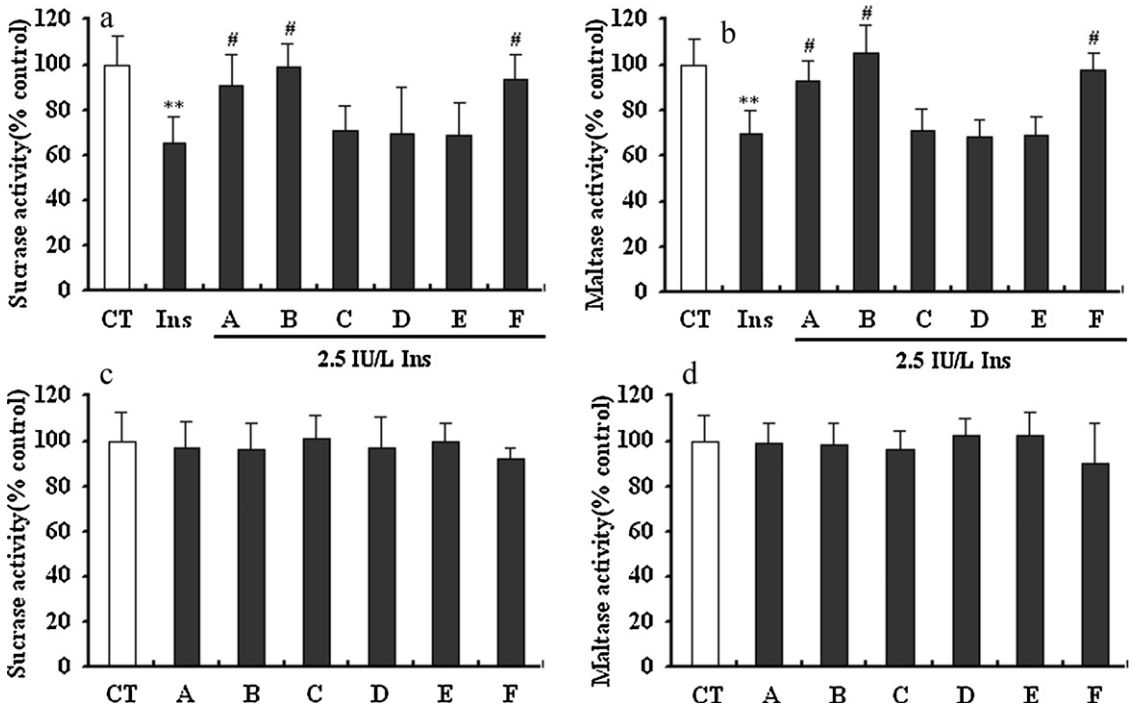

Fig. 6. Effects of pharmacological inhibitors including insulin receptor antibody (A, 0.2 μg/mL), I-OMe-AG538 (B, 10 μM), H-89 (C, 10 μM), LY294002 (D, 10 μM), chelerythrine (E, 10 μM) and PD-98059 (F, 10 μM) on sucrase (a and c) and maltase (b and d) activity with insulin (Ins, 2.5 IU/L) (a and b) or without insulin (c and d) in Caco-2 cells. The Caco-2 cells were cultured in medium containing test agents for 7 days. $^{**}p < 0.01$ vs. control cells (CT); $^{\#}p < 0.01$ vs. cells treated with Ins (2.5 IU/L) alone.

H-89, LY294002 and chelerythrine showed no effects on disaccharidase activities induced by insulin. These pharmacological inhibitors themselves showed no inhibitory effects on disaccharidase activities. These results suggested that the MAPK-dependent pathway may be involved in the inhibitory effects of insulin on disaccharidase activities and mRNA expression of SI complex. Some reports showed a crucial role of MAPK-dependent pathway in regulating the human intestinal epithelial differentiation and suppressing expression of SI complex [24,40]. Another report demonstrated existence of cross-talk between MAPK signal pathway and PI3K/Akt signal pathway for controlling of enterocyte differentiation [41]. It is well-known that the Ras/Raf/MEK/ERK cascade regulates intestinal epithelial cell proliferation. Previous studies showed that transfection of Caco-2 cells with an activated human Val-12 Ha-Ras gene repressed SI complex gene expression, suggesting that the Ras oncogene and its downstream effectors, which include ERK, may exert an antagonizing effect on regulation SI complex [42]. A recent study reported that the constitutive activation of the MEK/ERK cascade inhibited enterocyte differentiation, in part through Cdx2 phosphorylation resulting in inhibition of its transcriptional activity [43]. However, the present study showed that PD-98059 reversed the inhibitory effects of insulin on disaccharidase activities and mRNA expression of SI complex, which revealed that the MAPK-dependent pathway was involved in suppression of disaccharidase activities and mRNA expression of SI complex induced by insulin.

The Cdx2, belonging to the members of caudal-related homeobox gene family of transcription factors [44], has been confirmed the idea that it could be a central transcription factor in triggering epithelial differentiation in the intestine [45]. In addition, other transcription factors, namely GATA-4 and HNF-1α, are needed for the expression of intestinal differentiation-specific genes such as SI complex [46–48]. In cooperation with Cdx2, HNF-1α acts as a key factor on human intestinal cells to trigger the onset of their functional differentiation program including the expression of SI complex [49], whereas GATA-4 activates SI complex promoter activity requiring the presence of both HNF-1α and Cdx2 [50]. These findings imply a combinatory role of HNF-1α, Cdx2, and GATA-4 for regulation of SI complex transcription during development. It was also reported that the higher expression of Cdx2 was one of the reasons for increasing disaccharidase activities and expression in STZ-induced diabetic rats. However, the present study suggested that insulin may suppress the expression of SI complex via inhibiting the expression of the transcription factor Cdx2. A report showed that MAPK appears to be a potential activator of Cdx2 and inhibition of MAPK signals may inbihit binding of Cdx2 to the SI promoter [40]. Our results showed that insulin suppressed the expression of Cdx2 mRNA in Caco-2 cells and the suppression may be almost blocked by inhibitor of MAPK signal pathway PD-98059. The alteration in levels of Cdx2 mRNA was in line with that of SI complex mRNA. The results indicated that insulin suppressed mRNA expression of SI complex via affecting MAPK-dependent expression of Cdx2 mRNA. The real relationship between the MAPK-dependent pathway and the inhibitory effects of insulin on disccharidase activities and mRNA expression of SI complex is still obscure and requires further investigation. However, this study still provided direct evidences that the inhibitory effects of insulin on disccharidase activities and mRNA expression of SI complex were partly via the MAPK-dependent pathway.

In conclusion, the present study clearly showed that diabetes mellitus increased intestinal disaccharidase activities, accompanied by high mRNA and protein expression of SI complex. Insulin treatment reversed the increases induced by diabetes. The cellular results showed that insulin suppressed disaccharidase activities and down-regulated SI complex and Cdx2 mRNA expression in a concentration-dependent manner. The inhibitor of MAPK signal pathway PD-98059 abolished the suppression of disaccharidase activities and expression of SI complex and Cdx2 mRNA induced by insulin. All these findings revealed that insulin deficiency accounted for abnormal increase in intestinal disaccharidase activities and expression under diabetic states. Insulin plays an essential role in regulation disaccharidase activities and expression, at least in part, via the MAPK-dependent pathway.

Conflict of interest

The authors declare that they have no conflict of interest.

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