

Different sucrose-isomaltase response of Caco-2 cells to glucose and maltose suggests dietary maltose sensing

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Using the small intestine enterocyte Caco-2 cell model, sucrose-isomaltase (SI, the mucosal α -glucosidase complex) expression and modification were examined relative to exposure to different mono- and disaccharide glycemic carbohydrates. Caco-2/TC7 cells were grown on porous supports to post-confluence for complete differentiation, and dietary carbohydrate molecules of glucose, sucrose (disaccharide of glucose and fructose), maltose (disaccharide of two glucoses α -1,4 linked), and isomaltose (disaccharide of two glucoses α -1,6 linked) were used to treat the cells. qRT-PCR results showed that all the carbohydrate molecules induced the expression of the SI gene, though maltose (and isomaltose) showed an incremental increase in mRNA levels over time that glucose did not. Western blot analysis of the SI protein revealed that only maltose treatment induced a higher molecular weight band (Mw ~245 kDa), also at higher expression level, suggesting post-translational processing of SI, and more importantly a sensing of maltose. Further work is warranted regarding this putative sensing response as a potential control point for starch digestion and glucose generation in the small intestine.

Key Words: maltose, mucosal α -glucosidases, small intestine, sensing, sucrose-isomaltase

Chronic consumption of high glycemic foods and the accompanying quick high blood glucose response after food consumption is thought to be unhealthy, because it increases oxidative stress in cells and stresses the incretin system responsible for controlling blood sugar levels.⁽¹⁾ This increase in stress may correlate to Type 2 diabetes and coronary heart disease, and has been implicated as a factor in the development of obesity.⁽²⁾ Glycemic carbohydrate absorption occurs in the form of the monosaccharides glucose, fructose, and galactose; through digestion at the small intestine mucosal enterocyte level of maltooligosaccharide products of α -amylase hydrolysis of starch and the disaccharides of sucrose and lactose. Maltose is one of the major starch degradation products of α -amylase. Starches are digested at different rates in the human small intestine, mainly depending on food form; enterocytes in the more distal parts may not require constant luminal-surface presence of the α -glucosidases for their conversion to glucose. The ability of small intestinal enterocytes to sense maltooligosaccharides for the purpose of digestion has not been established.

Digestion of starch in the human involves the function of several enzymes. Salivary and pancreatic α -amylases are secreted, respectively, to initiate and degrade starch to maltooligosaccharides.⁽³⁾ Pancreatic α -amylase plays a more dominant role in starch

digestion than salivary amylase.⁽⁴⁾ α -Amylase is an endo-enzyme (or endo-glucosidase) that hydrolyzes the internal α -1,4 glycosidic linkages of amylose and amylopectin to form short linear (i.e., maltose, maltotriose, maltotetraose) and branched dextrin chains (α -limit dextrins).⁽⁵⁾ These maltooligosaccharide products are further digested to glucose by the mucosal α -glucosidases, sucrose-isomaltase (SI) and maltase-glucoamylase (MGAM).⁽⁶⁾ All four catalytic subunits of SI and MGAM are capable of hydrolyzing α -1,4 linkages, while the isomaltase subunit has the capacity to hydrolyze the α -1,6 linkage.⁽⁷⁾ Mammalian small intestines have more abundant SI than MGAM,⁽⁸⁾ although the latter has higher enzyme activity.⁽⁹⁻¹¹⁾ The human Caco-2 cell line synthesizes only SI; though, because it contains the full complement of α -glucogenic activities, it is still considered a good model to study aspects of starch digestion.

Delivery of monosaccharides to the body depends on the expression of the monosaccharide transporters. After carbohydrate digestion to monosaccharides, glucose and galactose are transported into small intestinal enterocytes through the sodium-dependent glucose transporter (SGLT1); and fructose is transported into enterocytes by the facilitated glucose transporter type 5 (GLUT5). These monosaccharides are transported from enterocytes into the circulation system through the facilitated glucose transporter type 2 (GLUT2).⁽¹²⁾ GLUT2 also migrates to the apical surface to assist in fructose and glucose absorption when high sugar concentration is present.⁽¹³⁾ Understanding the coordination between the expression of the digestive enzymes and expression of the different types of transporters may provide another clue as to the control of glycemic response. Kishi⁽¹⁴⁾ showed in rats that fructose increased mRNA expression of SI which is one of the major α -glucosidases for hydrolysis of sucrose, and as well increased mRNA expression of SGLT1, GLUT5, and GLUT2. Thus, there may be coordination at the transcriptional level of both the digestion enzymes and glucose transporters. On the other hand, post-translational processing of SI involving different glycosylation steps adds another layer of complexity to regulation of the active form of the enzyme. Also, there are a number of types of carbohydrates that are exposed to the small intestinal enterocytes during the digestion and absorption processes. Le Gall *et al.*⁽¹⁵⁾ demonstrated that sugar (sucrose) sensing by the enterocytes is a complex process which combines the sensing of sugar, the sensing of sugar metabolites, and where the sensing receptors are located

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on the membrane.

Caco-2 cells are cells derived from human colonic adenocarcinoma cells. These cells are commonly used in *in vitro* models to study intestinal absorption of compounds. This cell line undergoes spontaneous differentiation and forms a monolayer after long-term culture and displays several biochemical and morphological characteristics of mature small intestinal enterocytes after the differentiation.⁽¹⁶⁾ Several clones have been isolated from the Caco-2 cell line and characterized for their activities. Among them, the Caco-2/TC7 clone was isolated from a late passage of the parental Caco-2 line and consists of a more homogeneous population with better representative functions of the small intestinal enterocytes, and with more developed intercellular junctions.⁽¹⁷⁾ Because it is known that Caco-2/TC7 cells express numerous transporters such as SGLT1, GLUT1, GLUT2, GLUT3, GLUT5, and CAT1, this cell line is used as a model of the absorptive properties of the intestinal mucosa.⁽¹⁸⁾

In this study, the sucrase-isomaltase and transporter transcription and translation response of human enterocyte Caco-2/TC7 cells to some commonly encountered simple glyceic carbohydrate structures was investigated. The focus was on whether the cells are able to sense the starch degradation disaccharide (maltose) that it is required to digest to glucose for absorption.

Materials and Methods

Caco-2 cell culture. The Caco-2/TC7 clone at passage of 82–93 was selected for this study. Cells were seeded as 1.5×10^3 cells/well on 96-well solid supports (Becton Dickinson, Franklin Lakes, NJ), 6.4×10^4 cells/well on 6-well solid support (Corning, Lowell, MA), or 1.25×10^5 cells/well on 6-well porous (0.4 μ m polycarbonate membrane) transwell supports (Corning, Lowell, MA). Cells were grown in Dulbecco's modified Eagle's medium (25 mM, or equal to 4.5 g/l glucose DMEM, Lonza BioWhittaker, Walkersville, MD) supplied with 10% heat-inactivated (56°C, 30 min) fetal bovine serum (FBS) (Lonza, Walkersville, MD), 50 μ g/ml gentamycin sulfate (J R Scientific Inc., Woodland, CA), 10 mM HEPES, 100 μ g/ml streptomycin and 100 U/ml penicillin (Lonza, Walkersville, MD), and 100 μ M non-essential amino acid (Lonza BioWhittaker, Walkersville, MD). Cells were incubated at 37°C with 5% CO₂, 95% air atmosphere, and at constant humidity. Because Caco-2/TC7 cells express higher levels of SI, SGLT1, GLUT1, GLUT2, and GLUT5 in the stationary phase of growth,⁽¹⁹⁾ cells required longer time culture. Media were renewed every 48 h until 10 days post-confluence for the complete differentiation of the cells.⁽¹⁵⁾ Cell viability was measured by the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium] assay.⁽²⁰⁾

Sugar digestion and transportation. Cells on transwells were washed twice with 1 ml PBS after they reached 100% confluence and were fully differentiated. Cells were then fed with glucose-free DMEM (Gibco, Carlsbad, CA) in the basolateral well region. Test carbohydrates of glucose and maltose were supplied to the glucose-free DMEM (Gibco, Carlsbad, CA). The different media were supplied to the apical part of the transwell. The amount of glucose in the media in apical and basolateral regions was measured at different incubation times using the glucose oxidase/peroxidase assay kit (GOPOD, Megazyme, Bray, Ireland).

Total RNA isolation. Total RNA of cells was stabilized by storing in RNAlater solution (Ambion, Austin, TX) at 4°C for overnight. Equipment used for RNA extraction was soaked in DEPC-H₂O (diethyl dicarbonate, 0.1% v/v; Sigma, St Louis, MO) for at least 1 h at 37°C followed by autoclaving at 121°C for 30 min to inactivate RNases. Total RNA was extracted using the SV Total RNA Isolation System (Promega, Madison, WI) according to manufacturer's protocol. RNA samples were diluted and measured at 260 and 280 nm. RNA concentration was calculated by A260 according to the Beer-Lambert law and RNA purity

was determined by the ratio of A260/A280. High purity of RNA should give an A260/A280 ratio value higher than 1.8.

cDNA synthesis and real-time PCR analysis. Relative levels of SI and SGLT1, GLUT5, and GLUT2 transporter mRNA expressed in the treated Caco-2 cells were quantified over a 48 h period by reverse transcription and qRT-PCR. Oligo dT (500 μ g/ml, Promega, Madison, WI) was used as primers for first-strand cDNA synthesis of total RNA. Reverse transcription polymerase chain reaction (qRT-PCR) was performed using two systems. Some samples were reverse transcribed by using the Access qRT-PCR System (Promega, Madison, WI) according to manufacturer's instructions. Each qRT-PCR (20 μ l) of other samples was performed by heating a mixture (1 μ l of Oligo dT, 1–2 μ g of total RNA, 1 μ l of 10 mM dNTP mix, Invitrogen, Carlsbad, CA, and 16 μ l of DEPC-H₂O) at 65°C for 5 min and then adding M-MuLV Reverse Transcriptase Reaction Buffer (BioLabs, Ipswich, MA) and 25 units of M-MuLV reverse transcriptase (BioLabs, Ipswich, MA) to react at 42°C for 1 h. The reaction was inactivated by heating at 95°C for 5 min.

qRT-PCR analysis was performed by using the MX3000P QPCR system (Stratagene, La Jolla, CA) in the presence of SYBR-green. The primers used were:

- SGLT1 forward primer (5'-TGGCAATCACTGCCCTTTA-3') and SGLT1 reverse primer (5'-TGCAAGGTGTCCGTGTAAT-3'),⁽¹⁵⁾
- GLUT2 forward primer (5'-GTCCAGAAAGCCCCAGATACC-3') and reverse primer (5'-GTGACATCCTCAGTT-CCTCTTAG-3'),⁽²¹⁾
- GLUT5 forward primer (5'-TCTCCTTGCAAACGTAG-ATGG-3') and reverse primer (5'-GAAGAAGGGCAGCA-GAAGG-3'),⁽¹⁵⁾
- SI forward primer (5'-CATCCTACCATGTCAAGAGCCAG-3') and reverse primer (5'-GCTTGTTAAGGTGGTCTGGTT-TAAAT-3'),⁽²⁵⁾
- β -Actin forward primer (5'-TCCTATGTGGGTGACGAGGC-3') and β -actin reverse primer (5'-CATGGCTGGGGTGT-GAAGG-3').⁽¹⁵⁾

The qRT-PCR reaction was performed using Brilliant II QPCR master mix (Stratagene, La Jolla, CA) scaled down to a 25 μ l per reaction. The reaction followed standard conditions and the melting temperature was 55°C. qRT-PCR results were expressed as fold change of relative amount of mRNA (User Bulletin of ABI Prism 7700 sequence detection system, 1997).⁽²³⁾

Total protein extraction. Cells were washed twice with 1 \times PBS (1 ml in apical compartment and 1 ml in basolateral compartment) and harvested in 1 ml of 1 \times ice cold PBS followed by centrifugation at 14,000 rpm for 3–5 min at 4°C. Contents of 2 to 3 wells were combined and centrifuged to increase the cell number in each microcentrifuge tube. After discarding the PBS supernatant, cell pellets were lysed on ice for 20 min using 50–60 μ l of lysis buffer consisting of 1% Nonidet P-40 (NP-40), 0.5% sodium deoxycholate, 150 mM NaCl, 20 mM Tris-HCl pH 8.0, 5% SDS, 10 mM DTT, and 1 mM PMSF. During the lysis process, cells were vigorously shaken every 5 min for complete lysis. Cells were then centrifuged at 14,000 rpm for 10 min at 4°C to remove cell debris. Protein concentration was measured using the BCA protein assay kit according to the manufacturer's protocol (Pierce, Rockford, IL). Cell lysates were denatured and stabilized by adding sample solvent (final concentration after adding together was 10% glycerol, 0.1% bromophenol blue, 2% SDS, 50 mM Tris-Cl pH 6.8, and 10 mM DTT) and placed in a boiling water bath for 5 min. Samples were then directly used for SDS-PAGE or stored at –80°C.

Western blot analysis. For Western blot analysis, equal amounts of protein-containing lysates (35 μ g) were loaded onto 8% SDS-PAGE mini-gels and separated by electrophoresing with 35 mA/gel constant current for 1 h. The separated proteins were then electro-transferred at 100 V to PVDF membranes (BioRad,

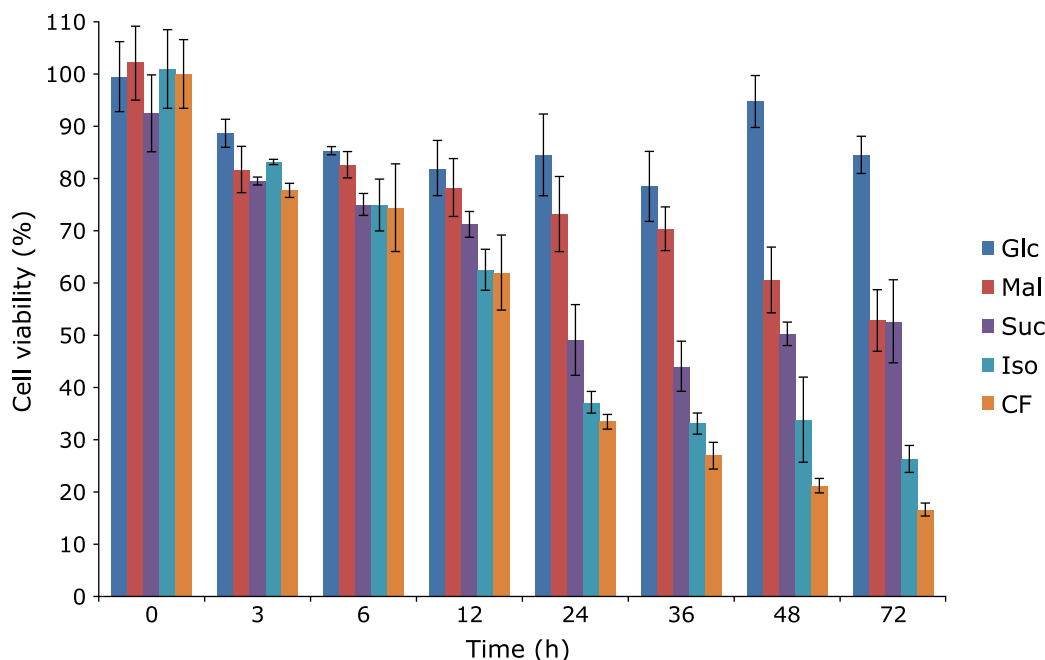


Fig. 1. Viability of Caco-2/TC7 cells at different times after being fed with carbohydrate-free media (CF) or the same media supplemented with 12.5 mM sucrose (Suc), 25 mM fructose (Fru), 25 mM glucose (Glc), 12.5 mM maltose (Mal), or 12.5 mM isomaltose (Iso).

Hercules, CA) at room temperature with ice in the transfer cassette for 1.5 h. The membranes were blocked with phosphate buffered saline containing 5% skim milk (BioRad, Hercules, CA) and 0.1% Tween 20 (BioRad, Hercules, CA) at room temperature for 1–2 h and then allowed to react with 1/1500 dilution of mouse anti-SI antibodies (B. Nichols, Baylor College of Medicine) (mixed with equal volume of HSI 1/691/79, HIS 3/190, and HIS 3/42/1/2; Quezada-Calvillo *et al.*, 2007).⁽⁸⁾ After incubation with horseradish-peroxidase-conjugated secondary antibody (1/2000 dilution, anti-mouse and anti-goat, from Santa Cruz Biotechnology, Santa Cruz, CA; anti-rabbit from Amersham, GE Healthcare Biosciences, Piscataway, NJ), proteins were visualized using enhanced chemiluminescence (ECL) (Pierce, Rockford, IL or Amersham of GE Healthcare Biosciences, Piscataway, NJ) followed by exposure to Amersham hyperfilm.

Western blot signals used for protein quantification were measured using ImageStudioLite software (LI-COR Biosciences, Lincoln, NE). Band intensities of SI with different treatments and at different incubation times were measured and quantified based on corresponding β -actin band intensity (SI band intensity/ β -actin band intensity).

Results

Caco-2/TC7 cell model. For carbohydrate digestion and absorption, the viability of Caco-2/TC7 cells on the different test carbohydrates was first investigated. Cells maintained a normal viability for two days when feeding was ≥ 12.5 mM glucose. When different carbohydrates were tested (equivalent with 12.5 mM glucose), cells fed by the glucose had a higher viability than those fed by disaccharides of maltose, sucrose and isomaltose. Among the disaccharides, maltose had the highest viability (Fig. 1). Although the viability measured by MTS showed lower values when disaccharides were the energy source, there was not a significant difference in number of viable cells and the total protein content (data not shown), indicating that cells cultured under these conditions maintained cell viability within the time duration of 2

days. Thus, the Caco2/TC7 line was determined appropriate as a model system to study the digestion and absorption of the test carbohydrates.

Carbohydrate digestion and absorption. Caco-2 cells absorbed and transported glucose, as well as fructose (not shown), to reach an apparent equilibrium between the apical and basolateral regions of the transwells (Fig. 2). After glucose concentration equilibrated, a gradual decrease in both apical and basolateral regions was likely due to sugar utilization by the cells. Maltose was digested as evidenced by a gradual increase in glucose in both the apical and basolateral regions. Therefore, SI functioned to hydrolyze maltose to glucose which was transported through the cell monolayer. Perhaps of note, a repeatable initial lag of ~ 2.5 h of appearance of glucose in both apical and basolateral sides was observed that could coincide with a recognition of the substrate and maturation of SI for its digestion.

Gene expression affected by carbohydrate treatment.

Following treatment of the Caco-2 cells with the different carbohydrates, expression of SI, SGLT1, GLUT5, and GLUT2 was measured at different time points using qRT-PCR. Expression levels were positive in all cases compared to that of carbohydrate-free media (Fig. 3). Notably, SI expression incrementally increased over 24 h after exposure to maltose, compared to glucose which did not over the same period; and at 48 h had opposite effects of decrease in the former increase in the latter. This suggests that the two sugar molecules were sensed differently by the enterocytes and, in the case of maltose, triggered SI expression perhaps related to the need for it to be digested. The α -1,6 linked isomaltose, which was only digested at a very low rate as evidenced by cell viability (Fig. 1) and digestion/absorption (not shown) data; showed a similar, though less pronounced, trend as maltose.

For the transporters, mRNA levels of SGLT1 went through a cycling pattern of an initial rise in expression followed by a reduction, and then again a rise. A difference was seen between glucose and maltose, as well as with isomaltose and fructose, with the latter producing a more delayed inflection point; perhaps due to

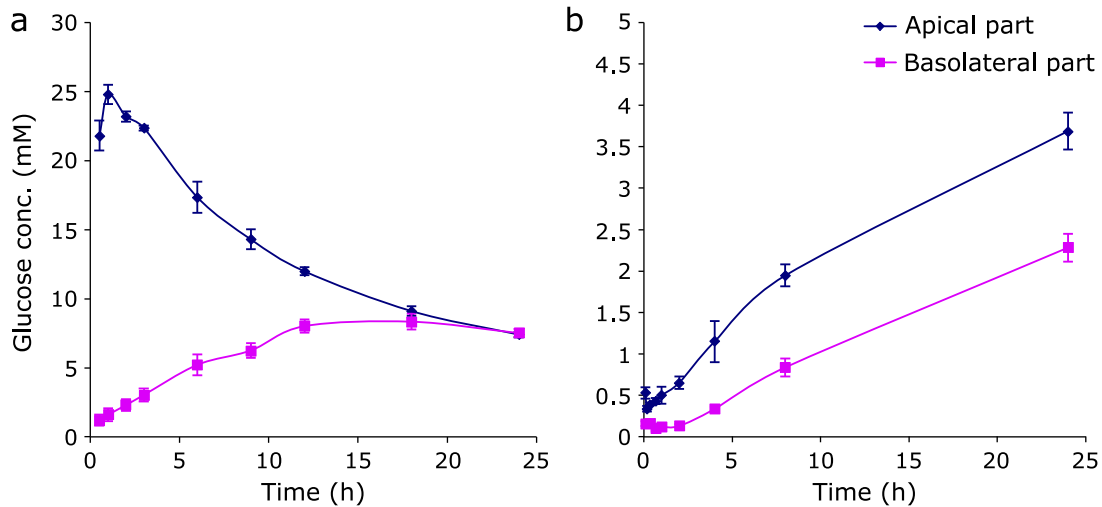


Fig. 2. Digestion (loss in the apical compartment) and absorption (gain in the basolateral compartment) through the Caco-2/TC7 monolayer of the monosaccharide glucose (a), compared to the disaccharide maltose (Glc α -1,4 Glc) (b).

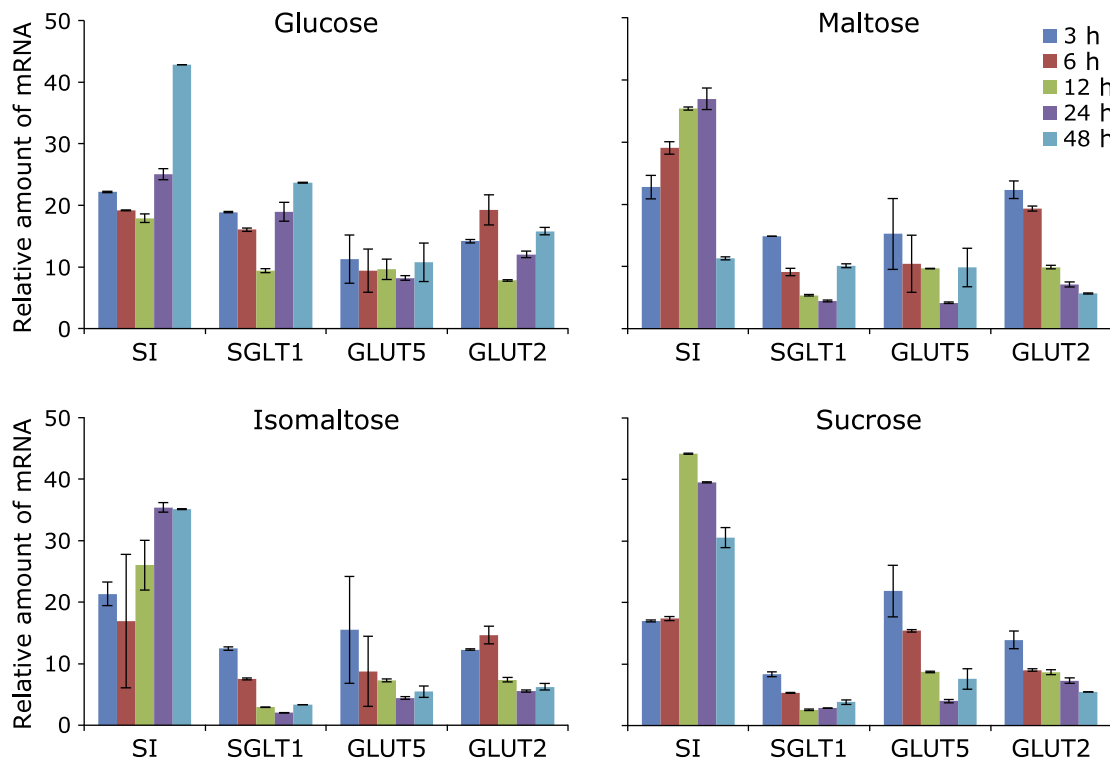


Fig. 3. qRT-PCR results of gene transcription of different carbohydrate transporters and SI. The data are presented as fold change of mRNA levels over the lowest mRNA expression of cells treated in carbohydrate-free media. Results represent the mean \pm SEM of at least three wells of treated Caco-2 cells.

lower glucose concentrations. GLUT5 gene expression was initially most affected by fructose, agreeing with its function in fructose transport. For GLUT2, which is mainly responsible for transporting the monosaccharide out to the basal-lateral side, all showed similar initial mRNA levels, and glucose showing a cycling pattern while the others a reduction in levels over time.

SI change in molecular weight induced by maltose.

Western blotting of SI in cells treated with the different carbohydrates showed that maltose alone caused a shift to higher molecular weight at 12 h and with a subsequent lowering of molecular

weight at 24 h (Fig. 4). Different concentrations of maltose were also used in the culture media, and a level of 12.5 mM was found to be the minimum level of maltose for the induction of the high molecular band of SI (not shown). This higher molecular band of SI appeared over the 250 kDa protein marker, compared to the lower SI molecular weight of 245 kDa suggesting post-translational modification. Immunoprecipitation with MALDI-TOF analysis of tryptic peptides verified that the higher molecular weight variant was SI (not shown).

Levels of SI in Western blots were notably higher with maltose

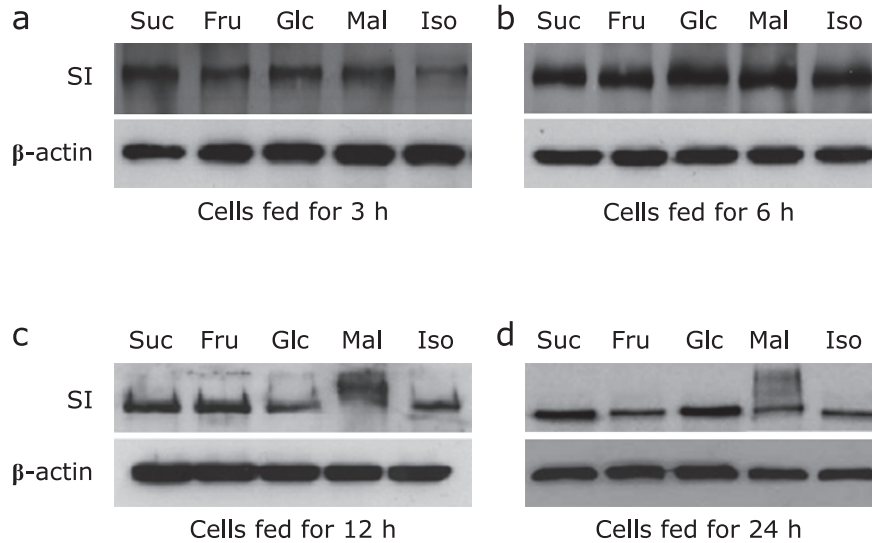


Fig. 4. Western blots of the sucrase isomaltase (SI) enzyme complex after feeding with different sugars for 12 h. Only maltose showed a shift in the band to a higher molecular weight species, which may be the active form of SI that can digest maltose.

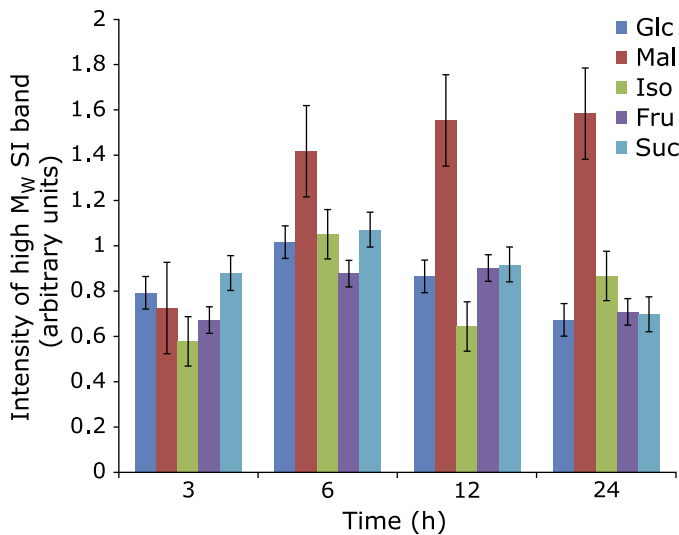


Fig. 5. Intensity of the high molecular weight SI band in Western blots reveals a higher protein expression level in Caco-2 cells treated with maltose compared to other sugars (mean \pm SD, $n = 3$).

treatment at 6, 12, and 24 h, than were SI levels for other treatments (Fig. 5). This supported qRT-PCR results where SI expression increased over time with maltose treatment.

Discussion

While small intestinal enterocyte monosaccharide (sugar) sensing has been known to alter metabolic events within the cell, including SI gene expression, and trigger gut hormone responses with systemic consequences,^(14,15,24) sensing of α -amylase starch degradation products (maltose or other maltooligosaccharides) by gut enterocytes with effect on SI has not been reported. In appetite studies, maltose sensing was recently reported at the site of the sweet taste receptor T1R2 and T1R3 subunits,^(25,26) however was not related to SI processing or maltooligosaccharide digestion. In

a way, the existence of an enterocyte sensing mechanism for starch degradation products would not be surprising given the importance of dietary glucose generation to the body. Dietary-derived glucose, versus that from glycogenolysis or gluconeogenesis, supplies a critical portion of glucose for metabolic processes. In an era of excess caloric intake in industrialized countries, and even in urbanized areas of developing countries, the body remains wired to efficiently digest starch and take up the glucose product.

Of the small intestine enterocyte α -glucosidases, SI and MGAM, Caco-2 cells only express SI. However, SI includes the activities of MGAM and is, thus, a good representative complex for human α -glucosidase activity.⁽²⁷⁾ SI is a highly glycosylated protein with N- and O-glycosylation sites that are added during co-translational and post-translational processing, as is similar to that of other glycoproteins,⁽²⁸⁾ before the final insertion of the enzyme in the microvillar membrane.⁽²⁹⁾ N- and O-glycosylation of SI are essential modifications that are associated with folding and intracellular trafficking.⁽³⁰⁾ SI is sorted to the apical membrane via O-linked glycans that mediate its association with lipid rafts or detergent-resistant membranes which is associated with increased SI activity.⁽³¹⁾

In the present study, only maltose treatment induced synthesis of a higher Mw SI protein band and MALDI-TOF and MS/MS analyses verified it to be SI. Expression level was higher for the new species of SI in cells treated with maltose compared to the other treatments. We speculate that this coincides with the final O-glycosylation step for trafficking SI to the lipid raft for subsequent maltose digestion. Thus, maltose, in contrast to other sugars tested, may act as a signal molecule to trigger SI processing and trafficking to the apical membrane.

The indication of maltose sensing in the current study has implications as another possible control point of starch digestion and gluconeogenesis. The data suggests that maltose may positively affect the post-translational processing of SI to mobilize and activate the enzyme at the enterocyte apical surface.

Conflict of Interest

No potential conflicts of interest were disclosed.

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