Carbohydrate Polymers 132 (2015) 41–49

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Enzymatic synthesis of 2-deoxyglucose-containing maltooligosaccharides for tracing the location of glucose absorption from starch digestion

Byung-Hoo Lee[a], Dong-Wan Koh[b], Paul R. Territo[c], Cheon-Seok Park[d], Bruce R. Hamaker[e,**], Sang-Ho Yoo[b,*]

[a] Department of Food Science & Biotechnology, College of BioNano Technology, Gachon University, Seongnam, Republic of Korea
[b] Department of Food Science & Technology, BK21+ Project Team, and Carbohydrate Bioproduct Research Center, Sejong University, Seoul, Republic of Korea
[c] Department of Radiology and Imaging Sciences, Indiana University, School of Medicine, Indianapolis, IN, USA
[d] Graduate School of Biotechnology, and Institute of Life Science and Resources, Kyung Hee University, Yongin, Republic of Korea
[e] Whistler Center for Carbohydrate Research and Department of Food Science, Purdue University, West Lafayette, IN, USA

ARTICLE INFO

Article history:
Received 23 March 2015
Received in revised form 2 June 2015
Accepted 4 June 2015
Available online 12 June 2015

Keywords:
2-Deoxyglucose (2-DG)
Amylosucrase
Glucose absorption
Maltooligosaccharides
Mucosal α-glucosidases

ABSTRACT

The ileal brake mechanism which induces a potentially beneficial slower gastric emptying rate and increased satiety is triggered by macronutrients including glucose from glycemic carbohydrates. For optimization of this diet-induced health benefit, there is the need for a way to determine the location of glucose deposition in the small intestine. Labeled 2-deoxyglucose (2-DG) can be used to trace the location of glucose absorption due to its accumulative property in the small intestine enterocytes. However, because pure glucose, or 2-DG, is directly absorbed in the proximal small intestine, we designed 2-DG containing maltooligosaccharides (2-DG-MOs) that can be used with a mild α-glucosidase inhibitor to attain an analytical method for determining location-specific delivery of glucose and its physiological effect.

1. Introduction

Starch is the major glycemic carbohydrate, as well as energy source, in the human diet (Gropper & Smith, 2012). After ingestion of starchy foods, starch molecules are hydrolyzed to glucose by the combined action of α-hydrolytic enzymes. The salivary and pancreatic α-amylases produce linear and branched oligosaccharides, which are then fully hydrolyzed in the small intestine to glucose by the combined action of the four mucosal α-glucosidases. The mucosal α-glucosidases consist of two complexes each with two catalytic enzymes, maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI) (Dahlqvist & Telenius, 1969). These four α-glucosidases, recognized as N- and C-terminal subunits (ntMGAM, ctMGAM, ntSI, and ctSI) are commonly known as maltase, glucoamylase, isomaltase, and sucrase, respectively (Jones et al., 2011; Quezada-Calvillo et al., 2008). All four α-glucosidases hydrolyze α-1,4 glycosidic linkages which are found in the linear portions of starch molecules, and sucrase and isomaltase show additional hydrolytic activities of α-1,2 on sucrose and α-1.6 on branches of starch. The liberated glucose is absorbed into the small intestine enterocytes *via* the Na^+-dependent glucose transporter 1 (SGLT-1) and transported out the basolateral membrane by glucose transporter 2 (GLUT-2), and on to the liver where it is metabolized (Fox, 2004). Glucose infused specifically at the distal part of the small intestine stimulates L-cells, and releases glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) hormones which affect both gastric emptying rate and satiety at the level of the hypothalamus (Maljaars, Peters, Mela, & Masclee, 2008; Wen, Phillips, Sarr, Kost, & Holst, 1995). Thus, the location of glucose absorption in the small intestine is a critical factor related to the triggering of these desirable physiological responses, and design of food ingredients to trigger the L-cells is a way to improve their health-related property. Materials, however, are needed to study locational digestion of dietary carbohydrates and the potential physiological effect.

2-Deoxyglucose (2-DG) is structurally similar to glucose, differing at the C-2 position, where the hydroxyl group is reduced to hydrogen (O'Neil, 2006). While glucose is absorbed into enterocyte cells *via* SGLT1, the 2-DG is absorbed *via* the GLUT2, which is

* Corresponding author. Tel.: +82 2 3408 3911; fax: +82 2 3408 3319.
** Corresponding author. Tel.: +1 765 494 5668; fax: +1 765 494 7953.
E-mail addresses: hamakerb@purdue.edu (B.R. Hamaker), shyoo@sejong.ac.kr (S.-H. Yoo).

http://dx.doi.org/10.1016/j.carbpol.2015.06.012
0144-8617/

less distributed at the apical surface of the cells (Ait-Omar et al., 2011; Au, Gupta, Schembri, & Cheeseman, 2002; Shirazi-Beechey, 1996). Once it enters the enterocyte, the absorbed 2-DG is rapidly phosphorylated by hexokinase to 2-deoxyglucose 6-phosphate (2-DG-6P) as is glucose itself (Newsholme & Carrié, 1994; Stümpel, Burcelin, Jungermann, & Thorens, 2001); however, 2-DG-6P will be slowly dephosphorylated by glucose 6-phosphothase with a 1000-fold slower rate than glucose 6-phosphate (Chi, Pusateri, Carter, Norris, McDougal Jr, & Lowry, 1987). Also, 2-DG-6P is not an efficient molecule for the glucose transporter systems (Gallagher, Fowler, Gutterson, MacGregor, Wan, & Wolf, 1978). Thus, the 2-DG-6P accumulates in the small intestinal enterocyte cells (Burant & Bell, 1992; Olefsky, 1978). Due to this accumulative property in the cell, labeled 2-DG's (*e.g.*, ^{14}C-, ^{3}H-, and 2-F-2-DG) are used to trace the location of glucose entry into a range of target cells using different types of imaging systems (Durham & Woolsey, 1985; Nedergaard & Astrup, 1986; Yamato, Kataoka, Mizuma, Wada, & Watanabe, 2009).

As an analytical tool to study the physiological effect of locational deposition of dietary glucose in the small intestine, 2-DG holds promise; however 2-DG itself is absorbed in the proximal part of the small intestine similar to glucose. To investigate the relationship between the location of digestion and physiological response, in the present work 2-DG was attached to a maltooligosaccharide with the idea that its digestion rate could be controlled using a technique of selective inhibition of the mucosal α-glucosidases which we have recently reported (Lee et al., 2012). To produce the 2-DG-maltooligosaccharides (2-DG-MOs), we used amylosucrase (AS; E.C. 2.4.1.4) with 2-DG as an acceptor molecule (Fig. 1). The AS catalyzes a consecutive transglucosyl reaction from sucrose onto the acceptor molecule, and produces α-1,4-linked α-glucans (Kim et al., 2013; Kim, Kim, & Yoo, 2015; Potocki de Montalk et al., 2000). The synthesized 2-DG-MOs were hypothesized to be hydrolyzed similarly to glucose by the mucosal α-glucosidases as they compare in size with homogenous maltooligosaccharides. ^{14}C-labeled 2-DG-containing synthesized products will be applied to test the correlation between the location of digestion and absorption, and physiological response *in vivo*.

2. Materials and methods

2.1. Materials

Sucrose and 2-DG were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Waxy corn starch (WCS) was a gift from Tate and Lyle, Inc. (Decatur, IL, USA). Chemical components for Luria-Bertani (LB) medium were obtained from Difco Laboratories (Detroit, MI, USA). Other chemical reagents were purchased from Sigma–Aldrich Chemical Co.

2.2. Production of recombinant amylosucrase from Neisseria polysaccharea

The *E. coli* BL21(DE3) transformant harboring recombinant AS gene from *Neisseria polysaccharea* (*Np*AS), which was prepared as previously described (Jung et al., 2009), was grown in LB broth with 20 μg of kanamycin per 1 mL at 37 °C until absorbance reached 0.6 at 600 nm. The cells were collected by centrifugation (5000 × *g* for 20 min at 4 °C) after isopropyl-β-D-thiogalactoside (IPTG) induction at 16 °C for 12 h, and the precipitant was resuspended in 50 mM Tris–HCl (pH 7.0). Nickel-nitrilotriacetic acid (Ni-NTA) affinity column chromatography (Qiagen, Hilden, Germany) was used to purify recombinant AS by increasing imidazole concentration (0–250 mM). The purified protein concentration was determined by the Bradford method (Bradford, 1976).

2.3. Determination of NpAS activity by DNS

An enzyme assay was carried out in pre-warmed 50 mM Tris–HCl buffer (pH 7.0) containing 0.1 M of sucrose and 1 mg/mL of WCS at 35 °C for 10 min (Kim et al., 2011). After the reaction, the amount of released fructose was measured by the dinitrosalicylic acid (DNS) method, using fructose as a standard (Sumner & Howell, 1935). One unit of AS was defined as the amount of enzyme that catalyzes the production of 1 μmol of fructose per min.

2.4. Production of 2-deoxyglucose containing maltooligosaccharides

Enzymatic synthesis of 2-DG-MOs was carried out in 50 mM Tris–HCl buffer (pH 7.0) containing 2-DG as an acceptor (3%, w/v) and sucrose (1 M) as a donor molecule. The pre-warmed substrate mixture was reacted at 35 °C at different times (0, 3, 6, 12, 24, and 24 h) with purified recombinant AS (5 U) to produce 2-DG-MOs. The enzyme reaction was terminated in a boiling water bath for 10 min.

2.5. Analysis of linear chain length distribution by HPAEC

Chain length distributions of enzyme-synthesized 2-DG-MOs were measured using a high-performance anion-exchange chromatograph (HPAEC) equipped with an electrochemical detector (ED40; Dionex, Sunnyvale, CA, USA). Filtered samples (with 0.22 μm, 25 μL) were injected onto a CarboPac PA-1 pellicular anion-exchange column (Dionex, Sunnyvale, CA, USA) and chromatographic separation of the linear oligosaccharides from the sample was achieved by gradient elution from 100% eluent A to 100% eluent B with 600 mM sodium-acetate in 150 mM sodium-hydroxide (Lee et al., 2007). Differences among the means of *number-average* degree of polymerization (DP_n) were evaluated using ANOVA and statistical significance was considered at $P < 0.05$.

2.6. MALDI-TOF-MS analysis

Molecular mass spectrum of 2-DG-MOs was obtained by Voyager DESTR matrix-assisted laser desorption/ionization-time of flight mass spectrometer (MALDI-TOF-MS, PE BioSystems, Framingham, MA, USA) and a 2,5-dihydroxybenzoic acid (2,5-DHB) as a matrix. One microliter of sample (0.1–0.001 mg/mL) was mixed with 1 mL of matrix (0.1–5 pmol/mL) and dropped on a sample plate, which was followed by air-drying until homogeneous crystals formed (Park et al., 2007).

2.7. Hydrolysis properties by human pancreatic α-amylase and mammalian mucosal α-glucosidases

Hydrolysis property of the enzyme-synthesized 2-DG-MOs by human pancreatic α-amylase was investigated. The synthesized-products were solubilized in 10 mM PBS buffer (pH 6.9, 10 mg/mL, w/v) and reacted with human pancreatic α-amylase (500 U, Meridian Life Science, Inc., Saco, ME, USA) at 37 °C for 24 h (Lee et al., 2013). The product was hydrolyzed using each of four recombinant mammalian mucosal α-glucosidases. The mucosal α-glucosidases (ctMGAM, ntMGAM, ctSI, and ntSI) were prepared as described (Jones et al., 2011). Each mucosal α-glucosidase [1000 U, one unit (U) enzyme activity arbitrarily defined as the amount of enzyme that released 1 μg of glucose from 1% maltose per 10 min at 37 °C] was reacted with 1% (w/v) of 2-DG-MOs in 10 mM PBS buffer (pH 6.8) at 37 °C for 6 h. The amount of glucose released was analyzed by the glucose oxidase/peroxidase (GOPOD) method (Vasanthan, 2001).

For enzyme kinetic study of the hydrolysis of individual mucosal α-glucosidases on 2-DG-MO, each enzyme (5 μg/mL) was reacted

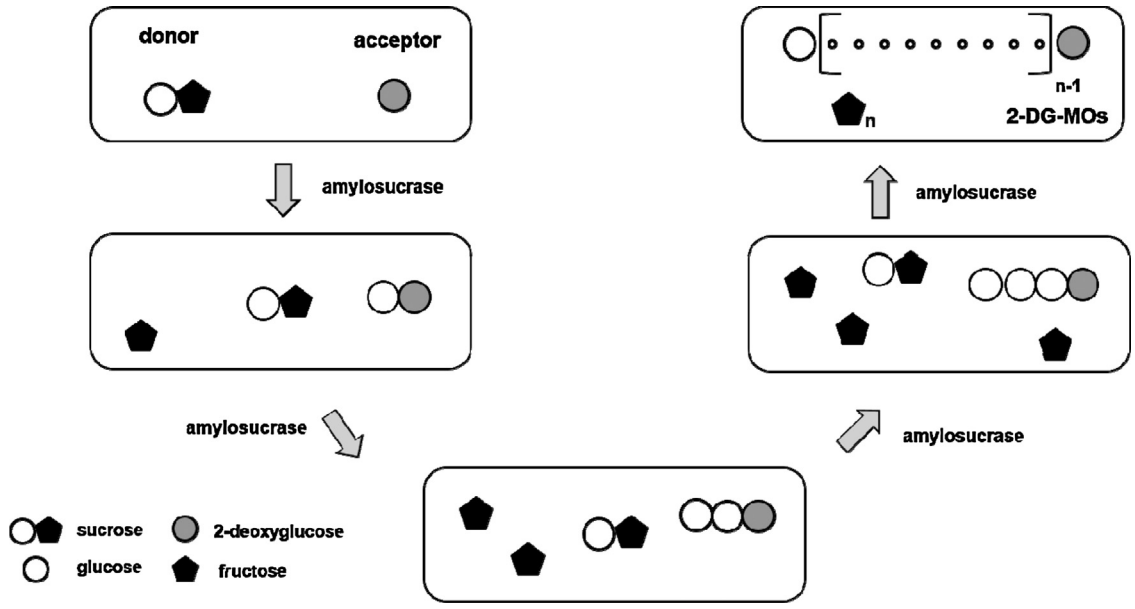

Fig. 1. Schematic of the action pattern of amylosucrase on 2-deoxyglucose as an acceptor to produce 2-deoxyglucose-containing maltooligosaccharides (2-DG-MOs).

with a range of sample concentrations (1–50 mg) for 60 min (ctMGAM: 30 min). Kinetic values, *apparent* K_m and V_{max}, were calculated using SigmaPlot 12 (Systat Software Inc., San Jose, CA, USA).

3. Results and discussion

Glycemic carbohydrates are hydrolyzed to glucose at different rates and at the different locations of the small intestine. Of particular interest is the distal digestion of glycemic carbohydrates and deposition of glucose in the ileum to trigger the ileal break and gut-brain axis mechanisms to reduce appetite and food intake. Tracing the location of carbohydrate digestion for optimization of these physiological effects is critical in designing food ingredients and processed foods that may be satiating and promote good health. Thus, the tools for tracing the location of carbohydrate digestion need to be developed. Here, we enzymatically synthesized novel 2-DG-MOs which can be utilized to trace the location of digestion in the gastrointestinal tract and to investigate its physiological response *in vivo*.

3.1. Production and identification of linear α-glucan from sucrose and 2-DG by transglycosyl property of NpAS

Fig. 2 presents the compositional changes in mono- and disaccharides after *Np*AS reaction (Fig. 2A), and the production pattern of elongated α-1,4 glucans (Fig. 2B) that occurs when 2-DG is used as an acceptor molecule for *Np*AS transglycosylating activity. *Np*AS acts by using sucrose as the donor molecule for the reaction to supply the glucose moiety to the acceptor molecule by hydrolysis of glycosyl linkage between glucose and fructose. Consequently, the amount of sucrose gradually decreased during the *Np*AS reaction while the amount of fructose, a hydrolyzed byproduct, increased. The amount of 2-DG also decreased, because it was used as the acceptor molecule to be linked by the glucose moiety generated from sucrose (Fig. 2A). In addition, a small amount of glucose was detected which was due to a minor hydrolysis property of *Np*AS instead of transferring activity (Pizzut-Serin et al., 2005; Skov et al., 2001). The amount of released glucose increased until 24 h; and decrease afterwards, because glucose was applied as the acceptor molecule (Mirza et al., 2001). As a result, a peak was detected at 9.5 min which had a different retention time compared to maltose (*data not shown*). The peak is thought to be a newly synthesized glucosyl-2-DG by the *Np*AS reaction, which also would be utilized as an acceptor molecule. This peak at 9.5 min of retention time showed the greatest signal intensity after 3 h *Np*AS reaction, then was decreased due to the active re-utilization as a glucosyl accepting molecule. Other disaccharide peaks formed in the AS reaction with sucrose were previously identified by other researchers as trehalulose (at 6.8 min) and turanose (at 8 min), which are sucrose isomers with α-1,1 and α-1,3 linkages (Potocki de Montalk et al., 2000; Wang et al., 2012).

As other researchers have shown, synthesized glucan chain lengths become longer as the reaction time increases (Fig. 2B) (Potocki-Veronese et al., 2005). Under this reaction condition, the maximal detectable DP was increased to over 30. As previously reported, it is expected that the chain length distribution of this newly synthesized elongated product can be controlled by simply changing initial sucrose concentration (Kim et al., 2011; Potocki de Montalk et al., 2000; Wang et al., 2012).

3.2. Identification of 2-DG-MOs produced by NpAS reaction

HPAEC analysis was used to show that 2-DG was incorporated into the MOs, and that the 2-DG-MO product had a different chemical structure from homogenous MOs (Fig. 3). The HPAEC elution time of the elongated 2-DG product compares to that of a homogenous α-1,4 linked glucose polymer which was produced under the same *Np*AS reaction conditions with glucose applied as an acceptor molecule. 2-DG-MO and homogenous MO peaks had different retention times implying their different monosaccharide compositions. The *number-average* degree of polymerization (DP_n) analysis, calculated from the HPAEC results, showed that the homogenous α-1,4 glucan had 10.4 ± 1.1 glucose units, and the 2-DG-containing MOs had 10.6 ± 0.1. The average chain length between the two products was not significantly different ($P < 0.05$), which suggests that *Np*AS did not discriminate glucose and 2-DG as acceptor molecules and resulted in a similar size distribution of α-glucans.

The molecular mass of 2-DG-MOs was determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (Table 1). The theoretical mass was calculated based on the molecular weights of glucose and 2-DG,

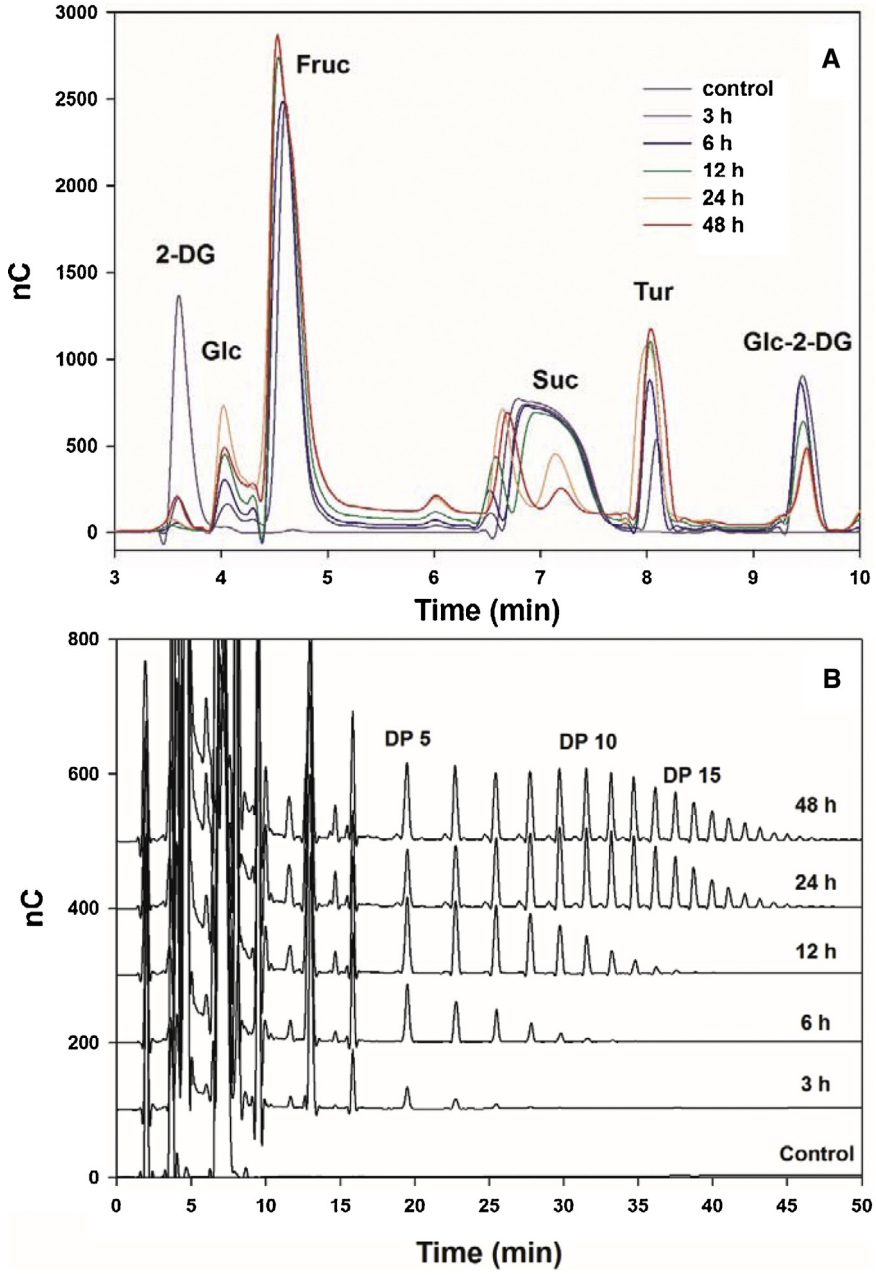

Fig. 2. Change of mono- and disaccharide composition during AS reaction with sucrose and 2-deoxyglucose (A) and increase in the number of glucosyl chains (B). 2-DG, 2-deoxyglucose; Glc, glucose; Fruc, fructose; Suc, sucrose; Tur, turanose; Glc-2-DG, glucosyl-2-deoxyglucose; DP, degree of polymerization; and nC, nanocoulomb.

the OH-group of the reducing end, number of glycosyl units (n), and sodium ion.

$$\text{Molecular mass of 2-DG-MOs} = 162(n-1) + 144 + 18 + 23 \text{ Da}$$
$$\text{Molecular mass of homogenous MOs} = 162n + 18 + 23 \text{ Da}$$

Both theoretical homogenous and 2-DG-MO masses increased by 162 Da per glucosyl unit added. 2-DG-MOs are 16 Da lower in size compared to homogenous MOs due to the absence of an oxygen atom from C2 hydroxyl group of glucose at the reducing end. Experimental results from MALDI-TOF-MASS confirmed that the mass was increased by 162 as a glucosyl unit was added, which corresponds to the theoretical mass of 2-DG-MOs. This result demonstrated that 2-DG-MOs were successfully produced by the action of AS.

Since 2-DG was successfully utilized as an acceptor molecule instead of glucose, this opens the possibility of producing various linear and branched structures of glucan materials that contain 2-DG using other types of glucosyltransferases combined with amylosucrase. For example, control of substrate concentration was shown previously to result in different sizes of linear α-glucans (Kim et al., 2011; Potocki-Veronese et al., 2005), and combination of other glycosyltransferases (*e.g.*, glycogen branching enzyme and/or 4-α-glucanotransferases) with amylosucrase generated various types of α-glucans such as glycogen or cycloamylose (Kim et al., 2011; Quanz, 2003). We expect that a range of α-glucan structures contain 2-DG, *e.g.*, α-1,6 linked structures, can be developed, which are designed to differ in digestion rate (Lee et al., 2013) to test physiological response based on digestion location in the small intestine.

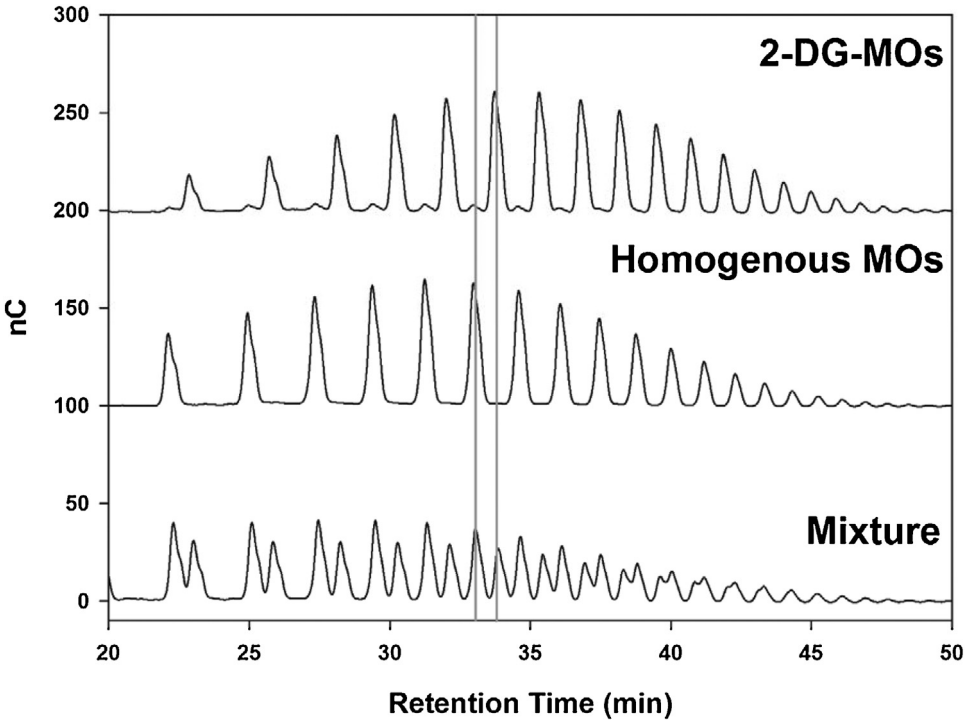

Fig. 3. High performance anion exchange chromatography (HPAEC) analysis of 2-DG and homogenous maltooligosaccharides, and their mixture. 2-DG-MOs, 2-deoxyglucose-containing maltooligosaccharides; homogenous MOs, homogenous maltooligosaccharides; and nC, nanocoulomb.

3.3. Hydrolysis product by α-hydrolytic enzymes

In the gastrointestinal tract, starch or starch-hydrolyzates are digested to glucose by a combination of α-hydrolytic enzymes, the two α-amylases and the four mucosal α-glucosidases. 2-DG-MOs must be completely hydrolyzed to monosaccharides by both sets of α-hydrolytic enzymes to trace the location of 2-DG absorption.

Fig. 4A shows the hydrolytic property of the 2-DG-MOs by human pancreatic α-amylase. 2-DG-MOs were mainly hydrolyzed to maltose (DP 2), and maltotriose (DP 3) which are major products of α-amylase digestion of starch (Jones et al., 1983). Also there were no remaining large oligosaccharides after the action of α-amylase (inset graph in Fig. 4A). Fig. 4B shows that the synthesized 2-DG-MO products were fully hydrolyzed by the mucosal α-glucosidases to its monosaccharides, 2-DG and glucose. Thus, full release of the 2-DG from the 2-DG-MOs was found which is required to trace the location of absorption (because the 2-DG will be absorbed and accumulated in the small intestinal enterocyte) (Kimmich & Randles, 1976). Following α-glucosidase digestion, there also were no remaining maltooligosaccharides (inset graph in Fig. 4B). Thus, the 2-DG-MOs will be hydrolyzed in the gastrointestinal tract and absorbed *in vivo*.

3.4. Hydrolysis rate of synthesized 2-DG-MOs by individual mucosal α-glucosidases

We also determined whether the synthesized product would be hydrolyzed at a similar rate to homogenous maltooligosaccharides. Table 2 shows the kinetic values including *apparent* K_m

Table 1
Theoretical molecular masses of 2-DG containing maltooligosaccharides and homogenous maltooligosaccharides, and molecular masses of 2-DG containing maltooligosaccharides by MALDI-TOF-MS.

DP[a]	Mass (Da)		
	Theoretical		Experimental
	2-DG containing glucan[b]	Linear glucan[c]	
6	997	1013	996
7	1159	1175	1159
8	1321	1337	1321
9	1483	1499	1483
10	1645	1661	1646
11	1807	1823	1809
12	1969	1985	1973
13	2131	2147	2133
14	2293	2309	2296
15	2455	2471	2459
16	2617	2633	2620

[a] DP: degree of polymerization.
[b] Calculated as $162(n-1)+144+18+23$ Da, due to the mass of a glucosyl residue, *n*DP, and 23 Da means the mass of sodium ion (Na^+).
[c] Calculated as $162n+18+23$ Da, 18 Da means the additional mass of a reducing end residue.

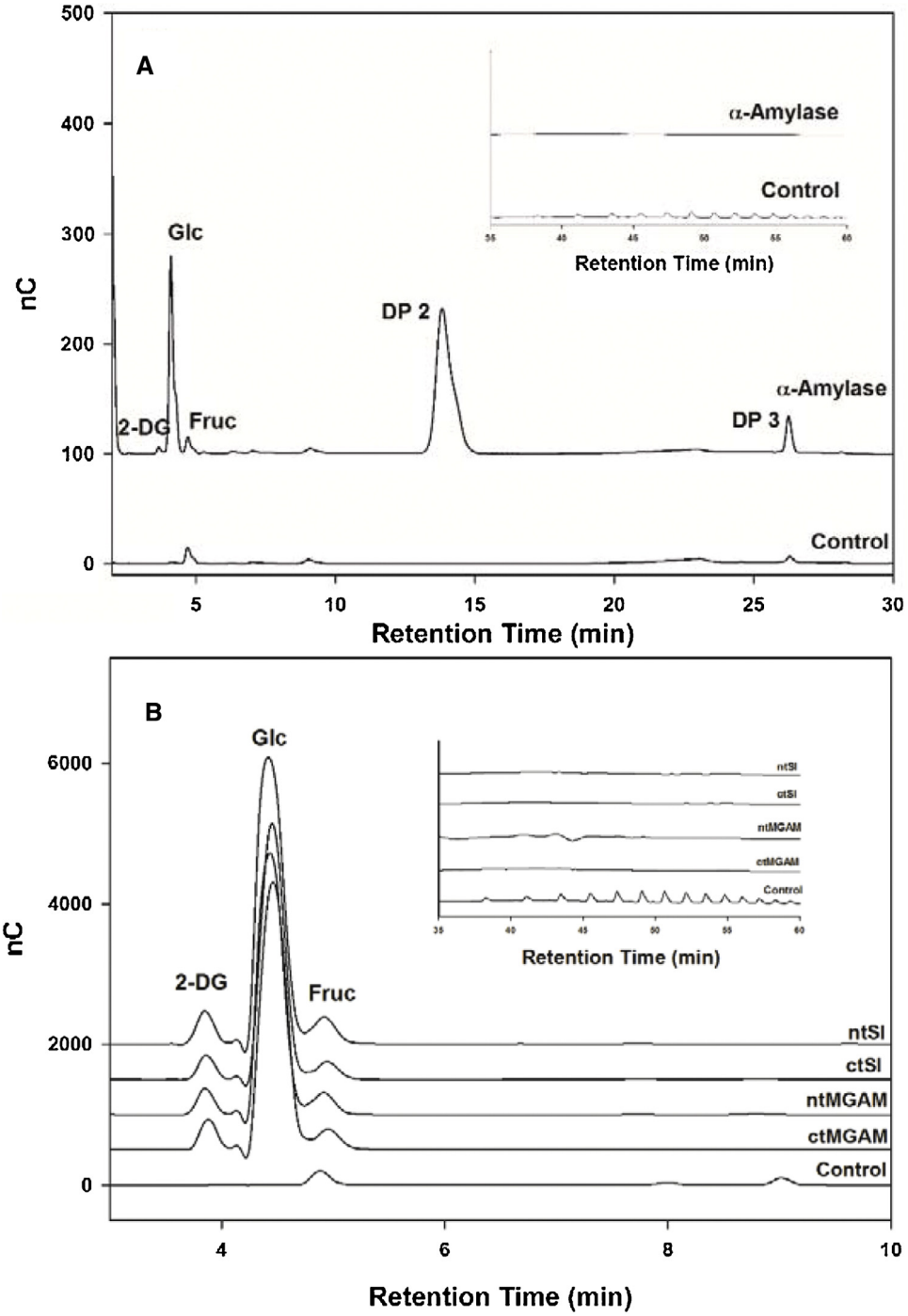

Fig. 4. Hydrolysis properties of 2-deoxyglucose-containing maltooligosaccharides (1%, w/v) using human pancreatic α-amylase (A), and the four individual recombinant mucosal α-glucosidases (B). The inset graphs in both panels indicate complete hydrolysis of larger oligosaccharides containing 2-DG. 2-DG, 2-deoxyglucose; Glc, glucose; Fruc, fructose; ctMGAM, C-terminal maltase-glucoamylase; ntMGAM, N-terminal maltase-glucoamylase; ctSI, C-terminal sucrase-isomaltase; ntSI, N-terminal sucrase-isomaltase; DP, degree of polymerization; and nC, nanocoulomb.

(mg/mL), *apparent* k_{cat} (s^{-1}) and k_{cat}/K_m when synthesized 2-DG-MOs and homogenous MOs were used as substrates. ctMGAM had the highest hydrolytic rate among the α-glucosidases for 2-DG-MO hydrolysis. Previous research showed that ctMGAM has higher activity on longer maltooligosaccharide (Heymann & Günther, 1994; Heymann, Breitmeier, & Günther, 1995; Lin et al., 2012), and in a confirmatory way our kinetics data showed that ctMGAM has 15–60 times higher hydrolytic rate compared other mucosal α-glucosidases during 2-DG-MO hydrolysis. Both homogenous and 2-DG-MOs had similar kinetic digestion values, and as they also had similar DP_n values, it can be concluded that the products are similarly hydrolyzed to monosaccharides by the action of the mucosal α-glucosidases.

Although ctMGAM has a higher hydrolytic rate compared other enzymes, it is inhibited by a high concentration of substrate which is known as the "brake effect", while ntMGAM is not

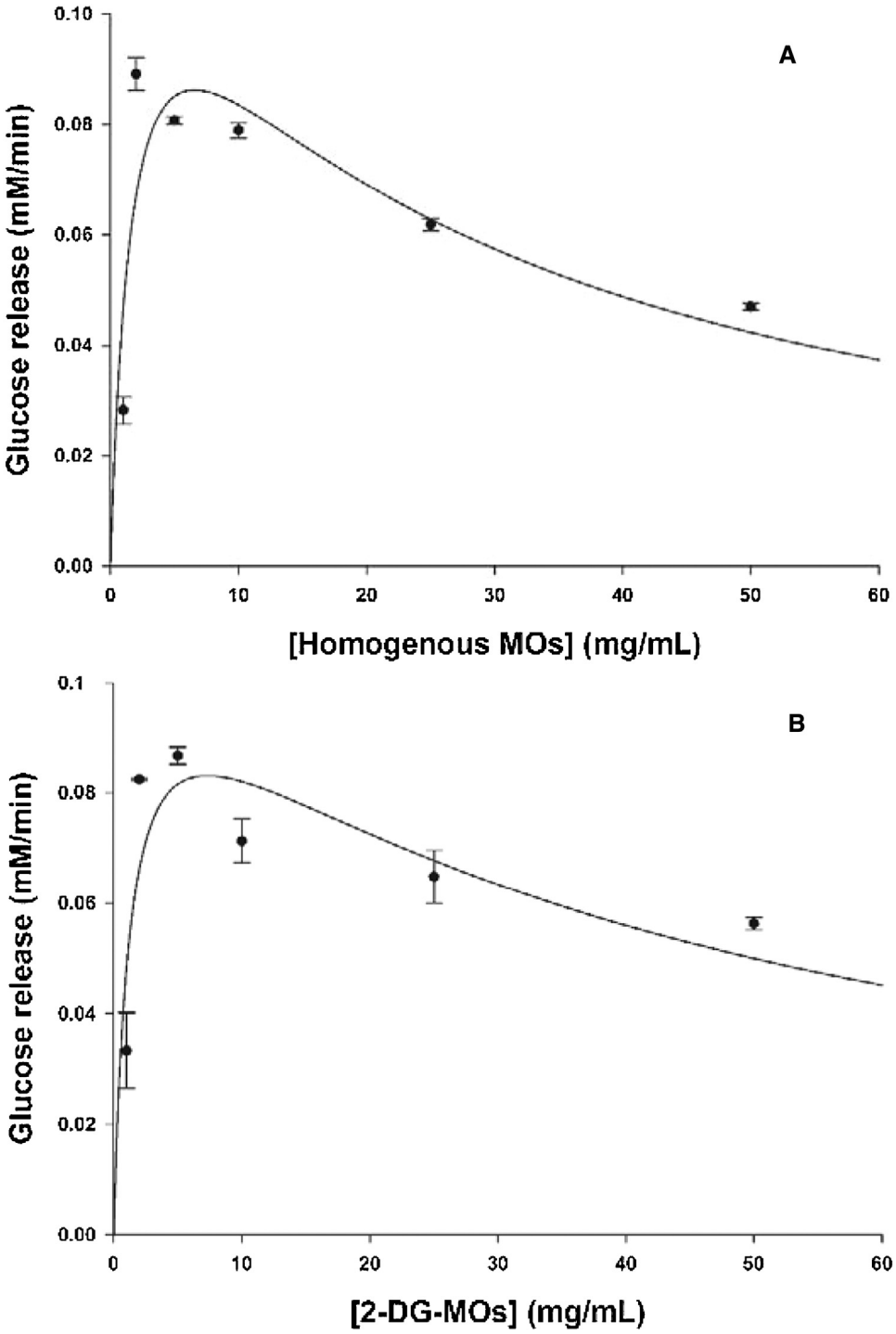

Fig. 5. Substrate inhibitory effect on homogenous maltooligosaccharides (A; homogenous MOs) and 2-deoxyglucose containing maltooligosaccharides (B; 2-DG-MOs) hydrolysis by C-terminal maltase-glucoamylase.

affected (Heymann & Günther, 1994; Quezada-Calvillo et al., 2007, 2008). Notably, ctMGAM showed a similar brake effect (Fig. 5) on 2-DG-MO hydrolysis. This result suggests that enzymatically synthesized products can be applied to *in vivo* tests for studying the maltooligosaccharide hydrolysis due to its similar hydrolytic properties.

The above results suggest that partial inhibition of ctMGAM could be applied to control the rate of glucogenesis of longer maltooligosaccharides. Such inhibition was demonstrated in a recent paper from our laboratory showing the possibility of "toggling" α-glucosidase activities by selectively inhibiting ctMGAM while leaving ntMGAM and ntSI uninhibited to continue glucogenesis at a relatively low rate (Lee et al., 2012). Thus, it is possible to control location of digestion in the small intestine of the 2-DG-MOs generated here by using such a strategy.

Table 2
Kinetic values of synthesized homogenous maltooligosaccharides (homogenous MOs) and 2-deoxyglucose containing maltooligosaccharides (2-DG-MOs) for individual recombinant mucosal α-glucosidases.

	ctMGAM (glucoamylase)	ntMGAM (maltase)	ctSI (sucrase)	ntSI (isomaltase)
Homogenous MOs				
Apparent K_m (mg/mL)	1.9 ± 1.1	14.0 ± 1.8	8.3 ± 2.0	14.5 ± 3.4
Apparent k_{cat} (s^{-1})	57.6 ± 15.8	33.0 ± 1.6	7.7 ± 0.6	7.3 ± 0.7
k_{cat}/K_m	31.3 ± 14.1	2.7 ± 1.0	1.0 ± 0.3	0.5 ± 0.2
2-DG-MOs				
Apparent K_m (mg/mL)	1.3 ± 0.7	18.6 ± 3.6	6.7 ± 1.9	20.3 ± 7.0
Apparent k_{cat} (s^{-1})	48.5 ± 9.8	26.8 ± 2.2	5.7 ± 0.5	6.9 ± 1.0
k_{cat}/K_m	36.8 ± 13.6	1.4 ± 0.6	0.9 ± 0.3	0.3 ± 0.15

4. Conclusions

Our results show that the synthesized 2-DG-MOs can be applied as a traceable material for location of digestion of glycemic carbohydrates and glucose deposition, to determine dosage effect, and for the design of ingredients and whole foods to induce the ileal break and gut-brain axis mechanisms to control appetite and food intake. 2-DG-MOs and homogeneous MOs were similarly hydrolyzed by the action of the individual α-hydrolytic enzymes (α-amylase and four mucosal α-glucosidases). With inhibition of specific mucosal α-glucosidases, such as by using a low concentration of acarbose (Lee et al., 2012), the location of 2-DG-MO hydrolysis would be extended to the distal part of the small intestine. Thus, this product, and other branched structures with labeled 2-DG that might be made, can be applied as a novel material to trace the location of glucose absorption from starch digestion related to physiological response.

Acknowledgements

This work was partially supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2012R1A2A2A01047497). This research was also supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (No. 2014M3A7B4051898). We are also thankful for support from the Whistler Center for Carbohydrate Research.

References

Ait-Omar, A., Monteiro-Sepulveda, M., Poitou, C., Le Gall, M., Cotillard, A., Gilet, J., et al. (2011). GLUT2 accumulation in enterocyte apical and intracellular membranes: A study in morbidly obese human subjects and ob/ob and high fat–fed mice. *Diabetes*, *60*(10), 2598–2607.

Au, A., Gupta, A., Schembri, P., & Cheeseman, C. I. (2002). Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. *Biochemical Journal*, *367*(1), 247–254.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1/2), 248.

Burant, C. F., & Bell, G. I. (1992). Mammalian facilitative glucose transporters: Evidence for similar substrate recognition sites in functionally monomeric proteins. *Biochemistry*, *31*(42), 10414–10420.

Chi, M. M. Y., Pusateri, M. E., Carter, J. G., Norris, B. J., McDougal, D. B., Jr., & Lowry, O. H. (1987). Enzymatic assays for 2-deoxyglucose and 2-deoxyglucose 6-phosphate. *Analytical Biochemistry*, *161*(2), 508–513.

Dahlqvist, A., & Telenius, U. (1969). Column chromatography of human small-intestinal maltase, isomaltase and invertase activities. *Biochemical Journal*, *111*(2), 139–146.

Durham, D., & Woolsey, T. A. (1985). Functional organization in cortical barrels of normal and vibrissae-damaged mice: A (3H) 2-deoxyglucose study. *Journal of Comparative Neurology*, *235*(1), 97–110.

Fox, S. I. (2004). *Human Physiology*. New York, NY: McGraw-Hill College.

Gallagher, B. M., Fowler, J. S., Gutterson, N. I., MacGregor, R. R., Wan, C.-N., & Wolf, A. P. (1978). Metabolic trapping as a principle of radiopharmaceutical design: Some factors responsible for the biodistribution of [18F] 2-deoxy-2-fluoro-D-glucose. *Journal of Nuclear Medicine*, *19*(10), 1154–1161.

Gropper, S. S., & Smith, J. L. (2012). *Advanced Nutrition and Human Metabolism*. Belmont, CA: Wadsworth Cengage Learning.

Heymann, H., Breitmeier, D., & Günther, S. (1995). Human small intestinal sucrase-isomaltase: Different binding patterns for malto- and isomaltooligosaccharides. *Biological Chemistry Hoppe-Seyler*, *376*(4), 249–253.

Heymann, H., & Günther, S. (1994). Calculation of subsite affinities of human small intestinal glucoamylase-maltase. *Biological Chemistry Hoppe-Seyler*, *375*(7), 451–455.

Jones, B. J., Brown, B. E., Loran, J. S., Edgerton, D., Kennedy, J. F., Stead, J. A., et al. (1983). Glucose absorption from starch hydrolysates in the human jejunum. *Gut*, *24*(12), 1152–1160.

Jones, K., Sim, L., Mohan, S., Kumarasamy, J., Liu, H., Avery, S., et al. (2011). Mapping the intestinal alpha-glucogenic enzyme specificities of starch digesting maltase-glucoamylase and sucrase-isomaltase. *Bioorganic & Medicinal Chemistry*, *19*(13), 3929–3934.

Jung, J.-H., Seo, D.-H., Ha, S.-J., Song, M.-C., Cha, J., Yoo, S.-H., et al. (2009). Enzymatic synthesis of salicin glycosides through transglycosylation catalyzed by amylosucrases from *Deinococcus geothermalis* and *Neisseria polysaccharea*. *Carbohydrate Research*, *344*(13), 1612–1619.

Kim, B.-S., Kim, H.-S., Hong, J.-S., Huber, K. C., Shim, J.-H., & Yoo, S.-H. (2013). Effects of amylosucrase treatment on molecular structure and digestion resistance of pre-gelatinised rice and barley starches. *Food Chemistry*, *138*(2/3), 966–975.

Kim, B.-S., Kim, H.-S., & Yoo, S.-H. (2015). Characterization of enzymatically modified rice and barley starches with amylosucrase at scale-up production. *Carbohydrate Polymers*, *125*(0), 61–68.

Kim, J.-H., Wang, R., Lee, W.-H., Park, C.-S., Lee, S., & Yoo, S.-H. (2011). One-pot synthesis of cycloamyloses from sucrose by dual enzyme treatment: Combined reaction of amylosucrase and 4-α-glucanotransferase. *Journal of Agricultural and Food Chemistry*, *59*(9), 5044–5051.

Kimmich, G., & Randles, J. (1976). 2-Deoxyglucose transport by intestinal epithelial cells isolated from the chick. *The Journal of Membrane Biology*, *27*(1), 363–379.

Lee, B.-H., Eskandari, R., Jones, K., Reddy, R. K., Quezada-Calvillo, R., Nichols, B. L., et al. (2012). Modulation of starch digestion for slow glucose release through toggling of activities of mucosal α-glucosidases. *The Journal of Biological Chemistry*, *287*(38), 31929–31938.

Lee, B.-H., Yan, L., Phillips, R. J., Reuhs, B. L., Jones, K., Rose, D. R., et al. (2013). Enzyme-synthesized highly branched maltodextrins have slow glucose generation at the mucosal α-glucosidase level and are slowly digestible *in vivo*. *PLoS ONE*, *8*(4), e59745.

Lee, C.-K., Le, Q.-T., Kim, Y.-H., Shim, J.-H., Lee, S.-J., Park, J.-H., et al. (2007). Enzymatic synthesis and properties of highly branched rice starch amylose and amylopectin cluster. *Journal of Agricultural and Food Chemistry*, *56*(1), 126–131.

Lin, A. H.-M., Nichols, B. L., Quezada-Calvillo, R., Avery, S. E., Sim, L., Rose, D. R., et al. (2012). Unexpected high digestion rate of cooked starch by the ct-maltase-glucoamylase small intestine mucosal α-glucosidase subunit. *PLoS ONE*, *7*(5), e35473.

Maljaars, P. W. J., Peters, H. P. F., Mela, D. J., & Masclee, A. A. M. (2008). Ileal brake: A sensible food target for appetite control. A review. *Physiology & Behavior*, *95*(3), 271–281.

Mirza, O., Skov, L. K., Remaud-Simeon, M., Potocki de Montalk, G., Albenne, C., Monsan, P., et al. (2001). Crystal structures of amylosucrase from *Neisseria polysaccharea* in complex with D-glucose and the active site mutant Glu328Gln in complex with the natural substrate sucrose. *Biochemistry*, *40*(30), 9032–9039.

Nedergaard, M., & Astrup, J. (1986). Infarct rim: Effect of hyperglycemia on direct current potential and [14C]2-deoxyglucose phosphorylation. *Journal of Cerebral Blood Flow & Metabolism*, *6*(5), 607–615.

Newsholme, E. A., & Carrié, A. L. (1994). Quantitative aspects of glucose and glutamine metabolism by intestinal cells. *Gut*, *35*(1 Suppl.), S13–S17.

O'Neil, M. J. (2006). *The Merck Index*. Whitehouse Station, NJ: Merck & Co., Inc.

Olefsky, J. M. (1978). Mechanisms of the ability of insulin to activate the glucose-transport system in rat adipocytes. *Biochemical Journal*, *172*(1), 137–145.

Park, J.-H., Kim, H.-J., Kim, Y.-H., Cha, H., Kim, Y.-W., Kim, T.-J., et al. (2007). The action mode of *Thermus aquaticus* YT-1 4-α-glucanotransferase and its chimeric enzymes introduced with starch-binding domain on amylose and amylopectin. *Carbohydrate Polymers*, *67*(2), 164–173.

Pizzut-Serin, S., Potocki-Véronèse, G., van der Veen, B. A., Albenne, C., Monsan, P., & Remaud-Simeon, M. (2005). Characterisation of a novel amylosucrase from *Deinococcus radiodurans*. *FEBS Letters*, *579*(6), 1405–1410.

Potocki-Veronese, G., Putaux, J.-L., Dupeyre, D., Albenne, C., Remaud-Siméon, M., Monsan, P., et al. (2005). Amylose synthesized *in Vitro* by amylosucrase: Morphology, structure, and properties. *Biomacromolecules*, *6*(2), 1000–1011.

Potocki de Montalk, G., Remaud-Simeon, M., Willemot, R.-M., Sarçabal, P., Planchot, V., & Monsan, P. (2000). Amylosucrase from *Neisseria polysaccharea*: Novel catalytic properties. *FEBS Letters*, *471*(2/3), 219–223.

Quanz, M. (2003). *Genetically modified plant cells and plants with an increased activity of an amylosucrase protein and a branching enzyme*. Potsdam, DE: PlantTec Biotechnologie GmbH.

Quezada-Calvillo, R., Robayo-Torres, C. C., Ao, Z., Hamaker, B. R., Quaroni, A., Brayer, G. D., et al. (2007). Luminal substrate Brake on mucosal maltase-glucoamylase activity regulates total rate of starch digestion to glucose. *Journal of Pediatric Gastroenterology and Nutrition*, *45*(1), 32–43.

Quezada-Calvillo, R., Sim, L., Ao, Z., Hamaker, B. R., Quaroni, A., Brayer, G. D., et al. (2008). Luminal starch substrate Brake on maltase-glucoamylase activity is located within the glucoamylase subunit. *Journal of Nutrition*, *138*(4), 685–692.

Shirazi-Beechey, S. P. (1996). Intestinal sodium-dependent D-glucose co-transporter: Dietary regulation. *Proceedings of the Nutrition Society*, *55*(1B), 167L 178.

Skov, L. K., Mirza, O., Henriksen, A., De Montalk, G. P., Remaud-Simeon, M., Sarçabal, P., et al. (2001). Amylosucrase, a glucan-synthesizing enzyme from the α-amylase family. *The Journal of Biological Chemistry*, *276*(27), 25273–25278.

Stümpel, F., Burcelin, R., Jungermann, K., & Thorens, B. (2001). Normal kinetics of intestinal glucose absorption in the absence of GLUT2: Evidence for a transport pathway requiring glucose phosphorylation and transfer into the endoplasmic reticulum. *Proceedings of the National Academy of Sciences*, *98*(20), 11330–11335.

Sumner, J. B., & Howell, S. F. (1935). A method for determination of invertase activity. *The Journal of Biological Chemistry*, *108*, 51–54.

Vasanthan, T. (2001). Enzymatic quantitation of total starch in plant products. In R. E. Wrolstad, T. E. Acree, E. A. Decker, M. H. Penner, D. S. Reid, S. J. Schwartz, C. F. Shoemaker, D. M. Smith, & P. Sporns (Eds.), *Current Protocols in Food Analytical Chemistry*. Hoboken, NJ: John Wiley & Sons, Inc, pp. E2.2.1-E2.2.

Wang, R., Bae, J.-S., Kim, J.-H., Kim, B.-S., Yoon, S.-H., Park, C.-S., et al. (2012). Development of an efficient bioprocess for turanose production by sucrose isomerisation reaction of amylosucrase. *Food Chemistry*, *132*(2), 773–779.

Wen, J., Phillips, S. F., Sarr, M. G., Kost, L. J., & Holst, J. J. (1995). PYY and GLP-1 contribute to feedback inhibition from the canine ileum and colon. *American Journal of Physiology Gastrointestinal and Liver Physiology*, *269*(6), G945–G952.

Yamato, M., Kataoka, Y., Mizuma, H., Wada, Y., & Watanabe, Y. (2009). PET and macro- and microautoradiographic studies combined with immunohistochemistry for monitoring rat intestinal ulceration and healing processes. *Journal of Nuclear Medicine*, *50*(2), 266–273.