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Preparation and characterization of maltosyl-sucrose isomers produced by transglycosylation of maltogenic amylase from *Bacillus stearothermophilus*

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Abstract

To develop a new transfer product of sucrose, sucrose was modified to maltosyl-sucrose using the transglycosylation activity of maltogenic amylase from *Bacillus stearothermophilus* (BSMA). The transglycosylation reaction was conducted with maltotriose and sucrose as the donor and acceptor, respectively. The presence of various sucrose transfer products was confirmed by thin layer chromatography (TLC) and high performance anion exchange chromatography (HPAEC). The sucrose transfer products were isolated by alkali-degradation followed by charcoal column chromatography using 20% (v/v) ethanol, then purified by ion exchange and Biogel P-2 gel permeation chromatographies. The structures of the major transfer products were determined to be 6^G-α-maltosyl-sucrose (maltosyl-sucrose **1**) and 6^F-α-maltosyl-sucrose (maltosyl-sucrose **2**) by LC-MS and ^{13}C NMR. The mixture of maltosyl-sucrose **1** and **2** showed low sweetness, high hygroscopicity, low Maillard reactivity, and high acid and heat stability. Furthermore, it had an inhibitory effect on mutansucrase and water-insoluble glucan formation. These results indicated that the mixture of maltosyl-sucrose **1** and **2** is a suitable sugar substitute useful for various food products.

Keywords: *Bacillus stearothermophilus* maltogenic amylase (BSMA); Transglycosylation; Sucrose; Maltosyl-sucrose

1. Introduction

The development of new sweeteners that are low in cariogenicity but which still retain the desirable

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features of sucrose has been in ever-increasing demand in the food industry. Using the intermolecular transglycosylation of cyclodextrin glucanotransferase (CGTase), 4^G-α-maltooligosyl-sucrose ("coupling sugar" is the commercial name) has been produced from a mixture of starch hydrolyzates and sucrose [1,2]. Cariogenicity tests in vitro and in vivo proved that this compound was definitely low in cariogenic activity [3]. Chiba et al. [4] reported on 6^F-α-D-glucosyl-sucrose produced by α-glucosidase from yeast [4]. It was also found that theanderose (6^G-α-D-glucosyl-sucrose) was produced as a transglycosylation product of sucrose by the *Bacillus* sp. SAM1606 α-glucosidase [5]. Commercially available sugar substitutes have been developed either as dietetic sweeteners or to meet the requirements of the food industry. β-Fructofuranosidase has been known to modify sucrose in the presence of xylose, isomaltose, and lactose, producing fructosylxyloside, isomaltosylfructoside, and lactosylfructoside (LacF), respectively [6]. These modified products of sucrose competitively inhibit the degradation of sucrose by glucosyltransferase (GTase) from *Streptococcus mutans* as an analogue to sucrose, and these products also reduce the formation of insoluble glucan. Furthermore, LacF was non-digestive but selectively utilized by bifidobacteria in the human intestinal bacteria flora [7].

Maltogenic amylases (MAases) are able to not only hydrolyze cyclodextrins (CDs) [8], but also catalyze the transglycosylation reaction in the presence of various acceptor molecules, consequently accumulating the transglycosylation products by forming α-(1,3), α-(1,4), and α-(1,6)-glycosidic linkages [9]. The transglycosylation activity of *Bacillus stearothermophilus* MAase (BSMA) has been utilized to produce the branched oligosaccharide (BOS) mixture [10] and to modify acceptor molecules such as neohesperidin [11], naringin [12], and sorbitol [13], thus creating transglycosylated molecules with improved characteristics and applications.

In the present study, sucrose was modified using the transglycosylation activity of BSMA. The resulting transfer products were purified, and their structures were determined by LC-MS and ^{13}C NMR. In addition, the physicochemical and physiological properties of the transfer products were also examined in vivo and in vitro.

2. Experimental

2.1. Materials

BSMA was obtained from recombinant *Escherichia coli* DH5α [*supE44*, *ΔlacU69* (*ϕ80lacZΔM15*), *hsdR17*, *recA1*, *endA1*, *gyrA96*, *thi-1*, *relA1*], which harbors the plasmid pSG18 [9]. This plasmid was constructed by inserting the BSMA gene originating from *B. stearothermophilus* KCTC0114BP into the *Hin*dIII site of pUC18. Cultivation of recombinant *E. coli* and purification of BSMA have been reported previously [9]. Invertase from baker's yeast and maltotriose were purchased from Sigma (St. Louis, MO, USA). Sucrose was purchased from Showa Chemical Co. (Fukuoka, Japan). All other chemicals used were of reagent grade.

2.2. Measurements of enzyme activity

An assay of the BSMA activity was conducted by the dinitrosalicylate (DNS) method, the procedure used for determining reducing sugars [14]. A mixture of 200 μl of 50 mM sodium citrate buffer (pH 6.0) and 250 μl of 1% (w/v) β-CD in the same buffer was prewarmed at 55 °C for 5 min. Next, an enzyme solution (50 μl) was added and incubated for 10 min. The reaction was stopped and colorized by adding 500 μl of the DNS solution (10.6 g of 3,5-dinitrosalicylic acid, 19.8 g of NaOH, 306 g of sodium potassium tartrate, 7.6 ml of phenol, 8.3 g of sodium metabisulfite, and 1416 ml of water), followed by boiling for 5 min. The absorbance of the mixture was measured by a spectrophotometer (Ultrospec III, Pharmacia LKB, Uppsala, Sweden) at 575 nm. One unit (U) of enzyme was defined as the amount producing 1 μmol of maltose per min.

2.3. Transglycosylation of sucrose

The reaction mixture—containing 10% (w/v) maltotriose, 20% (w/v) sucrose, and 10 U/ml BSMA in a 50 mM sodium citrate buffer (pH 6.0)—was incubated at 55 °C for 12 h. To stop the enzyme reaction, the reaction mixture was boiled for 10 min.

2.4. Analysis of transfer product

2.4.1. Thin layer chromatography (TLC)

A silica gel K5F TLC plate (Whatman, Kent, UK) was activated by placing it for 1 h in an oven set to 110 °C. Diluted samples of the reaction mixtures were spotted with a pipette on the silica gel plate, and the plate was placed in a TLC chamber containing a solvent mixture of acetonitrile/water (8:2 by volume) to be developed two times at room temperature. The developed plate was dried and visualized by dipping it in 0.3% (w/v) *N*-(1-naphthyl)-ethylenediamine and 5% (v/v) H_2SO_4 in methanol, followed by heating at 110 °C for 10 min [15].

2.4.2. High performance anion exchange chromatography (HPAEC)

The reaction mixture was centrifuged at 12,000 × *g* for 10 min, then filtered using a 0.45 μm membrane filter kit (Millipore, Bedford, MA, USA). Twenty microliters of sample was applied to a Dionex CarboPac PA100 column (0.4 cm × 25 cm) and eluted with a 0–15% (v/v) gradient of 600 mM sodium acetate and 150 mM sodium hydroxide at a flow rate of 1.0 ml/min.

2.5. Purification of the transfer products

The reaction mixture was set to pH 12 by adding sodium hydroxide, and the reducing sugars in the solution were decomposed by heating at 100 °C for 30 min [16]. The resulting solution (10 ml) was applied to a charcoal column (3.0 cm × 30 cm). The transfer products were washed with 2 l of 10% (v/v) ethanol and successively eluted with 1 l of 20% (v/v) ethanol. The eluate with 20% (v/v) ethanol was applied to an ion-exchange column (3.0 cm × 30 cm) to remove impurities from the reaction mixture. The eluate was concentrated at 60 °C using a rotary evaporator (EYELA, Tokyo, Japan). The pale-yellow syrup obtained by repeating this process was dissolved in distilled water. The concentrated sample was applied to a Biogel P-2 column (1.6 cm × 100 cm). The fractions containing transfer products were confirmed by TLC, collected, and lyophilized. The purified transfer products were used to examine physicochemical and physiological characteristics.

2.6. Structural analysis of transfer products

2.6.1. Liquid chromatography-mass spectrometry (LC-MS)

The LC-MS spectrum of the transfer products was obtained using a JEOL LC mate (Tokyo, Japan). The Atmospheric Pressure Chemical Ionization (APCI) mode was used. Five microliters of sample at a concentration of 100 mg/ml was directly injected into the instrument.

2.6.2. ^{13}C nuclear magnetic resonance (NMR) analysis

The ^{13}C NMR spectra of the major transfer products were recorded with a JEOL JNM LA-400 FT NMR spectrometer (Tokyo, Japan) in the mode for distortionless enhancement by polarization transfer (DEPT). The sample was dissolved in DMSO-d6 at 24 °C with tetramethylsilane as the internal reference. Collected ^{13}C NMR signals of the sample were assigned by comparing them with those of standard molecules.

2.6.3. Invertase treatment and preparative TLC

The transfer products were purified further by invertase treatment and preparative TLC for the structural determination. Each part of the TLC plate containing a different transfer product was cut off to isolate it from the plate. The transfer products were extracted with water, filtered, then concentrated. The concentrated sample was applied to a Biogel P-2 column to remove contaminated silica and binder compounds.

2.7. Physicochemical properties of sucrose transfer products

2.7.1. Hygroscopicity

Saturated salt solutions of $Mg(NO_3)_2{\cdot}6H_2O$ and $BaCl_2{\cdot}2H_2O$ were prepared in separate desiccators to achieve, respectively, 53 and 90% relative humidity (RH) at 25 °C. Each sample (maltosyl-sucrose, sucrose, maltose, BOS, and crystalline sucrose) was lyophilized under the following conditions: initial concentration, 50 mg/ml; temperature of drying, −50 °C; pressure, 10 mm Torr; and drying time, 2 days. Lyophilized samples (about 0.5 g each) were kept under 53% RH for 3 week to standardize them, then moved to a 90% RH chamber [17] and kept for 1

week to investigate the absorption of water vapor. The weight gain was measured as a percentage of moisture absorbed on the basis of the initial sample weight.

2.7.2. Maillard reactivity

McIlvaine buffers (1 ml each; pH 4 to pH 8 at intervals of 1) containing 0.5% (w/v) glycine and 10% (w/v) maltosyl-sucrose, sucrose, maltose, and glucose were heated at 100 °C for 60 min. The absorbance values at 420 (A_{420}) and 720 nm (A_{720}) for these solutions were measured after proper dilution with distilled water. The difference between the A_{420} and A_{720} values was used to show the Maillard reactivity. A McIlvaine buffer of each pH was prepared by mixing 0.1 M citric acid and 0.2 M Na_2HPO_4 [18].

2.7.3. Colorability

McIlvaine buffers (1 ml each; pH 4 or pH 7) containing 10% (w/v) of maltosyl-sucrose, sucrose, and maltose in screw-cap tubes were heated at 100–140 °C (at intervals of 10 °C) for 60 min in an air convection oven. As described earlier in the section on the Maillard reaction, the difference between the A_{420} and A_{720} values was used to show the colorability.

2.7.4. Heat and acid stability

Ten percent of each type of sugar (maltosyl-sucrose, sucrose, and maltose) in a McIlvaine buffer (1 ml each; pHs 4 or 7) contained in screw-cap tubes was heated at 100–130 °C (at intervals of 10 °C) for 60 min in an air convection oven. The saccharide remaining in each of these solutions was determined by HPAEC.

2.8. Sweetness

The relative sweetness of maltosyl-sucrose was determined by magnitude estimation [19]. Six trained panelists were presented with a 2% (w/v) sucrose solution (4 ml) as a reference. 1, 2, 4, and 8% (w/v) sucrose, and 2, 4, and 8% (w/v) maltosyl-sucrose solutions were served in paper cups randomly coded with three digit numbers at room temperature. Samples were assigned a sweetness score relative to the reference. To equate all scores, a normalization factor was determined for each judge and used to adjust each magnitude estimate. A number that made their grand mean equal to 10.0 was used to multiply the geometric means of the magnitude estimates given for various concentrations of the sugars. The magnitude estimates were converted into logarithms and expressed using the geometric mean. The dose/response curves for each sweetener were fitted to a power function.

2.9. Inhibition of mutansucrase and insoluble glucan synthesis

A crude mutansucrase made from *Streptococcus sorbrinus* NRRL 14555 was fractionated from the culture supernatant with a 50% saturation of ammonium sulfate, followed by dialysis against a 20 mM sodium phosphate buffer (pH 6.8). Sucrose (34 mM) and/or maltosyl-sucrose (34 mM) with 0.2 U/ml mutansucrase were/was incubated at 37 °C for 50 min. An aliquot (20 μl) was taken at 10 min intervals, and the reaction was stopped by adding 20 μl of 50 mM NaOH. The amount of fructose released was quantitatively determined on the TLC plate by densitometry (Bio-Rad, Hercules, CA, USA). The activity was determined based on the slope of the time course curve. One unit of mutansucrase was defined as the amount of enzyme that liberates 1 μmol of fructose in 1 min, using 34 mM of sucrose as the enzyme substrate.

The inhibitory effects of maltosyl-sucrose on the synthesis of water-insoluble glucan from sucrose by the activity of GTase from *S. sobrinus* was assayed. Pre-cultured *S. sobrinus* (2.5×10^6 ml^{-1}) was inoculated into 5 ml of a brain heart infusion (BHI) broth containing 50 mM sucrose and/or 50 mM maltosyl-sucrose, and then the bacterium was cultured at 37 °C for 24 h in a glass vial. The supernatants of individual reaction mixtures were discarded, and the synthesized glucan was washed with a buffer and dissolved in 0.5 M NaOH. Water-insoluble glucan was assayed by monitoring the absorbance at 550 nm.

2.10. Acid fermentation

The formation of acid from maltosyl-sucrose by *S. sorbrinus* was determined by measuring the pH of the culture broth. The bacteria were precultured, and the same amount of cells was added to new BHI broth containing 10 mM sucrose or maltosyl-sucrose. The pH of the aliquots was measured using a digital pH meter after 60 h incubation at 37 °C.

3. Results and discussion

3.1. Analysis of the transglycosylation products

TLC and HPAEC analyses were performed to examine the transglycosylation reaction of BSMA with maltotriose and sucrose. Fig. 1 shows the TLC analysis of the transfer products produced by the transglycosylation reactions with sucrose as an acceptor. Coupled with the HPAEC analysis, it was found that two major transfer products (**1** and **2**) and some unidentified transfer product (**3**) appeared in the transglycosylation reaction (Fig. 1, lane C). From the location in the TLC analysis and the retention time observed in the HPAEC analysis, it was assumed that the two major transfer products (**1** and **2**) were likely to be maltosyl-sucrose. About 30% of the donor (maltotriose) was utilized for the production of major transfer products **1** and **2**, based on the data from an HPAEC analysis of the reaction mixture. The molar ratio of product **1** to product **2** was about 1.1:1.

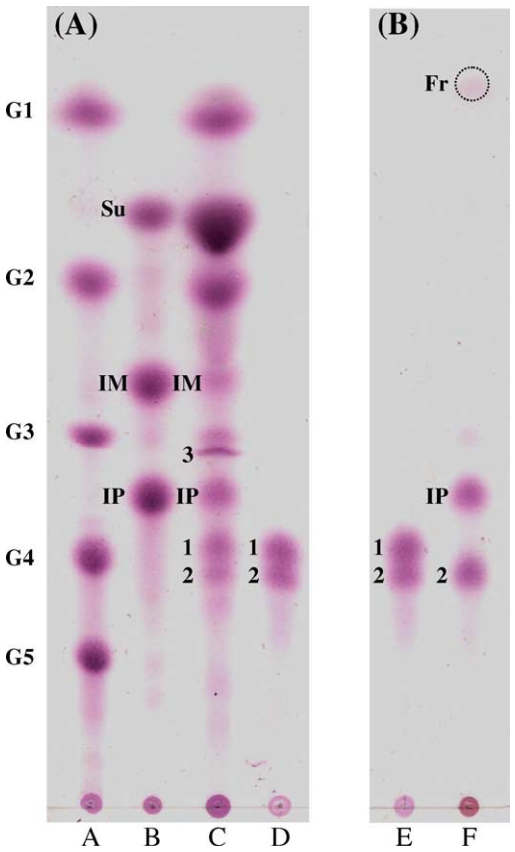

Fig. 1. TLC analysis of the sucrose transglycosylation products (A) and the change of transfer products with invertase treatment (B). (A) lane A: maltodextin standards; lane B: (IP) isopanose, (IM) isomaltose and (Su) sucrose; lane C: transfer products, (1) maltosyl-sucrose **1**, (2) maltosyl-sucrose **2**, (IP) isopanose and (3) an unidentified sucrose transfer product; lane D: purified major sucrose transfer products, (1) maltosyl-sucrose **1** and (2) maltosyl-sucrose **2**. (B) lane E: purified major sucrose transfer products, (1) maltosyl-sucrose **1** and (2) maltosyl-sucrose **2**; lane F: major sucrose transfer products treated with invertase, (2) maltosyl-sucrose **2**, (IP) isopanose and (Fr) fructose.

To remove the reducing sugars, the reaction mixture was boiled after adjusting the pH to an alkaline condition (pH 12). The transfer products were further purified by a series of charcoal, ion exchange, and Biogel P-2 gel permeation chromatographies. Finally, the two major transfer products **1** and **2** (maltosyl-sucrose **1** and **2**) were isolated (Fig. 1, lane D) and separated from each other. When the mixture was incubated with invertase, maltosyl-sucrose **1** was hydrolyzed to isopanose and fructose, indicating that the maltosyl unit is attached to the glucose moiety of sucrose via an α-(1,6)-glycosidic linkage. Treatment of the mixture with invertase results in the clear separation of compound **2** (Fig. 1, lane F). Maltosyl-sucrose **1** was purified by preparative TLC for further structural analysis.

3.2. Structural analysis of the transfer products by LC-MS and ^{13}C NMR

The molecular weights of purified transfer products **1** and **2** were determined by LC-MS (Fig. 2). One peak appeared in both compounds **1** and **2** at $m/z = 689$ ($[M + Na]^+$), which corresponded to the calculated molecular mass of sodium adducts of maltosyl-sucrose (666). The results imply that the major transfer products formed by BSMA were isomers of maltosyl-sucrose, because both compounds had the same molecular weight but different mobilities in TLC.

^{13}C NMR analyses were conducted to assign the glycosidic linkage between maltose and sucrose. Chemical shifts in the ^{13}C NMR spectra of maltosyl-sucrose were compared with those of sucrose. As shown in Fig. 3 and Table 1, a large chemical shift (bold-type figures in Table 1) was observed in maltosyl-sucrose. In the case of maltosyl-sucrose **1**, a shift occurred at C-6 in the glucose moiety of

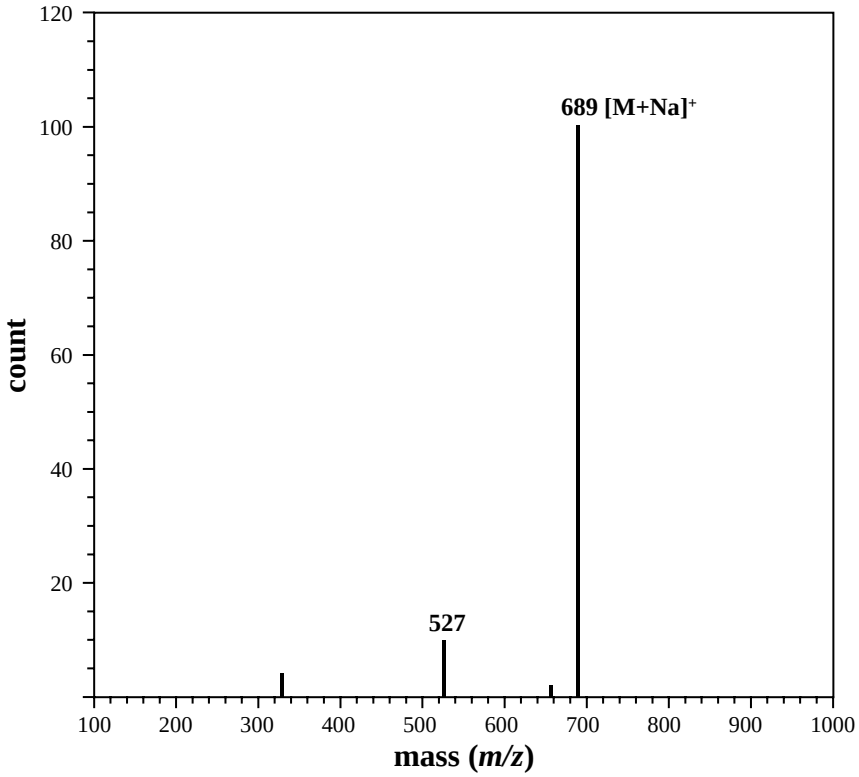

Fig. 2. Molecular weight determination of the purified maltosyl-sucrose **1** and **2** using LC-MS.

sucrose, from 60.9 to 66.9 ppm, whereas maltosyl-sucrose **2** exhibited a chemical shift from 63.1 to 68.9 ppm at C-6 in the fructose moiety of sucrose. In both maltosyl-sucrose **1** and **2**, significant chemical shifts (6.3 and 5.4, respectively) were observed at C-1 in the glucose moiety of maltose, indicating that the major transfer products **1** and **2** were 6^G-α-maltosyl-sucrose and 6^F-α-maltosyl-sucrose, respectively. The structures of **1** two sucrose acceptor products were also confirmed by the invertase treatment. Because it specifically cleaves the 2-β-fructofuranosyl linkage of sucrose, invertase hydrolyzed maltosyl-sucrose **1** to produce fructose and isopanose (**Fr** and **IP** in lane F of Fig. 1), whereas maltosyl-sucrose **2** was not hydrolyzed (**2** in lane F of Fig. 1). The results indicate that **1** (lane E of Fig. 1) has the chemical structure of a maltose molecule bound to the glucose moiety of sucrose by an α-(1,6)-glycosidic bond. Likewise, in maltosyl-sucrose **2**, maltose is connected to the fructose moiety of sucrose by an α-(1,6)-glycosidic linkage. CGTase and α-glucosidases transfer the glucose residue to sucrose to generate various sucrose transfer products by forming α-(1,4)- and α-(1,6)-glycosidic linkages, respectively [4,5]. Two sucrose transfer products, 6^G-α-maltosyl-sucrose and 6^F-α-maltosyl-sucrose, produced by BSMA are completely different from previously reported compounds in terms of their glycosidic linkages and the size of transfer molecules. The overall mechanism for the transglycosylation reaction by BSMA is depicted in Fig. 4, and a summary of the reaction of the invertase with the acceptor products of sucrose is described in Fig. 5.

3.3. Hygroscopicity of the sucrose transfer product mixture

To investigate the hygroscopicity of the mixture of transfer products **1** and **2**, the lyophilized samples were placed first in the desiccator equilibrated at 53% RH for 3 weeks, then stored at 90% RH for 1 week.

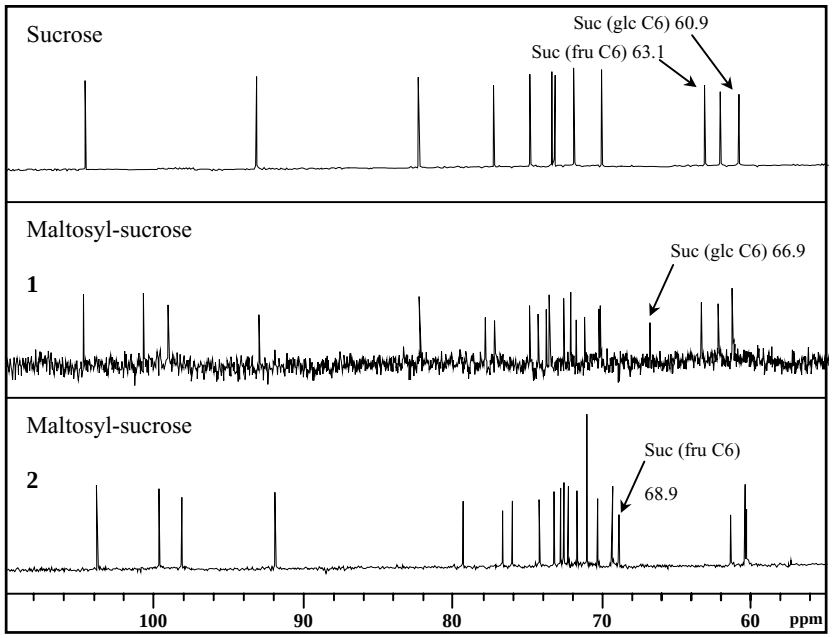

Fig. 3. ^{13}C NMR spectra of sucrose, and maltosyl-sucrose **1** and **2**.

Table 1
^{13}C NMR signals of maltose, sucrose, and maltosyl-sucrose 1, 2 (unit; ppm)

Ring	Carbon atoms	Maltose	Sucrose	Maltosyl-sucrose **1**	Differences	Maltosyl-sucrose **2**	Differences
Glc-I	1	100.4		100.6	0.2	99.6	0.8
	2	72.5		72.2	0.3	71.7	0.8
	3	73.7		73.8	0.1	72.8	0.9
	4	70.2		70.3	0.1	69.4	0.8
	5	73.5		73.6	0.1	72.6	0.9
	6	61.3		61.3	0.0	60.4	0.9
Glc-II	1	92.7		**99.0**	**6.3**	**98.1**	**5.4**
	2	72.1		72.1	0.0	71.0	1.1
	3	74.1		74.3	0.2	73.2	0.9
	4	77.8		77.8	0.0	76.7	1.1
	5	70.8		71.2	0.4	70.3	0.5
	6	61.3		61.3	0.0	60.4	0.9
Glc-A	1		92.9	92.9	0.0	91.8	1.1
	2		71.8	71.8	0.0	71.0	0.8
	3		73.3	73.5	0.2	72.6	0.7
	4		70.0	70.2	0.2	69.2	0.8
	5		73.2	72.6	0.6	72.3	0.9
	6		60.9	**66.9**	**6.0**	60.2	0.7
Fru-B	1		62.1	62.3	0.2	61.4	0.7
	2		104.5	104.6	0.1	103.7	0.8
	3		77.1	77.2	0.1	76.0	1.1
	4		74.8	74.9	0.1	74.2	0.6
	5		82.1	82.2	0.1	79.3	2.8
	6		63.1	63.4	0.3	**68.9**	**5.8**

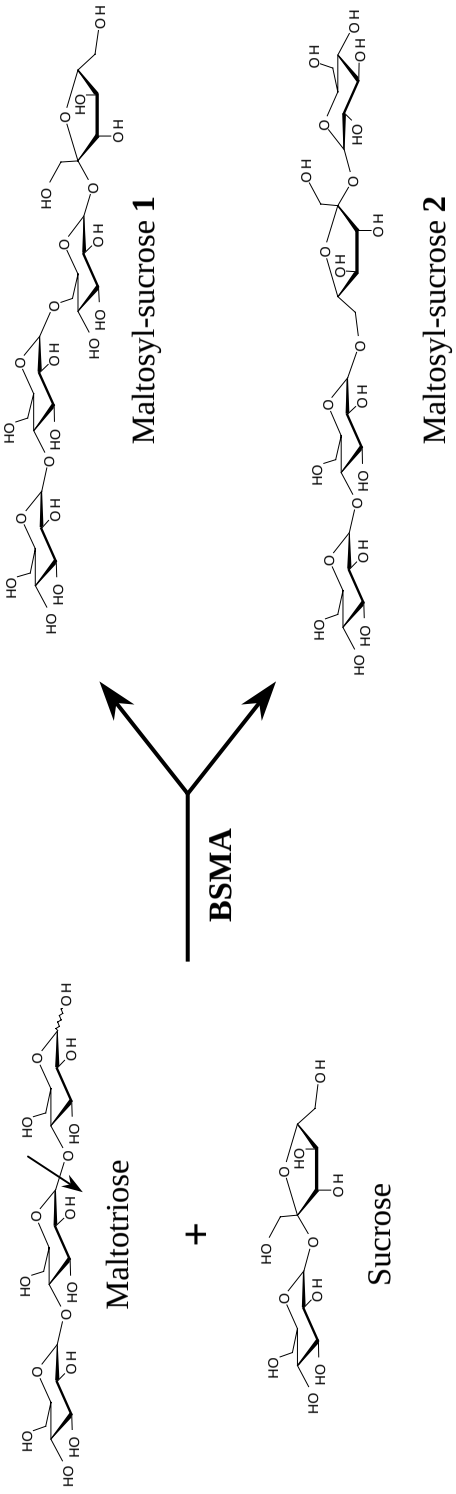

Fig. 4. Transglycosylation reaction of *Bacillus stearothermophilus* maltogenic amylase with sucrose as an acceptor.

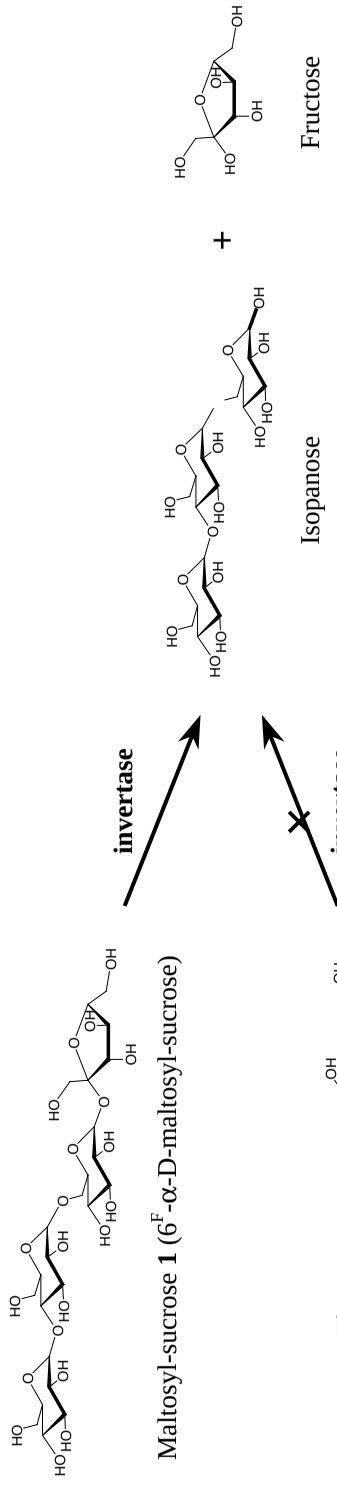

Fig. 5. Summary of the hydrolysis reaction of transfer products by invertase.

The weight gain of each sample was measured at a predetermined time intervals (24 h). The lyophilized maltosyl-sucrose mixture had greater ability to absorb moisture than sucrose and maltose when the mixture was stored at 90% RH. The hygroscopic properties of these oligosaccharides were in the order of maltosyl-sucrose, BOS, sucrose, and maltose (Fig. 6). Thus, the results confirmed that, compared to maltose and sucrose (DP 2), maltosyl-sucrose (degree of polymerization or DP 4) showed a higher moisture absorption power, and the results agreed well with those of Donnelly et al. [20].

The mixture of maltosyl-sucrose was not strongly colored by the Maillard reaction (Fig. 7A), suggesting that maltosyl-sucrose may be an excellent sugar substitute to be used when inhibition or control of the Maillard reaction is desired. In caramelization, the fragmentation of sugars occurs to a significant extent at acid pH and increases considerably at alkali pH. At pH 7, in contrast to pH 4, browning of maltosyl-sucrose increased with heating temperature (Fig. 7B). Regarding the caramelization of saccharide, a pH increase from 4 to 8 strongly enhanced the polymerization of the carbonyl compounds generated by the thermal degradation of saccharide, whereas at a higher pH value, the intermediates were almost equally transformed into brown polymers.

Compared with sucrose, the mixture of maltosyl-sucrose **1** and **2** showed relatively high thermostability. More than 50% of the maltosyl-sucrose mixture was stably maintained at 130 °C. However, maltosyl-sucrose **1** and **2** showed a different thermostability when tested separately (Fig. 8). Heating at 130 °C in acidic conditions caused a complete degradation of sucrose and maltosyl-sucrose **1**, but maltosyl-sucrose **2** was degraded by only about 28%. These results may be related to the thermoreactivity of the fructose residue in maltosyl-sucrose **1**.

3.4. Sweetness

The relationship between sweetness intensity and concentration for each sweetener was represented graphically on log–log coordinates (data not shown). The relative sweetness of maltosyl-sucrose was about 23% of that of sucrose. The sweetness of maltooligosaccharides decreased with an increase in DP. Therefore, it is reasonable to assume that maltosyl-sucrose is less sweet than sucrose.

3.5. Cariogenicity

S. mutans and *S. sobrinus* are known to be involved in dental caries in humans and experimental animals

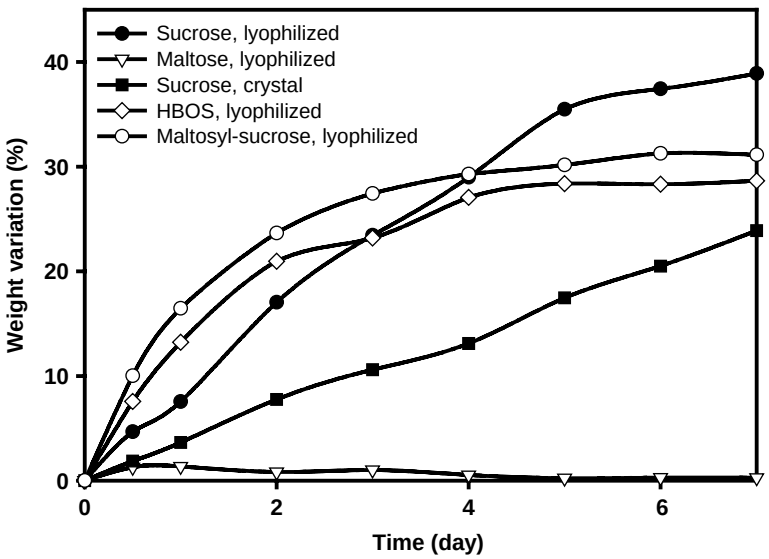

Fig. 6. Hygroscopicity of maltosyl-sucrose.

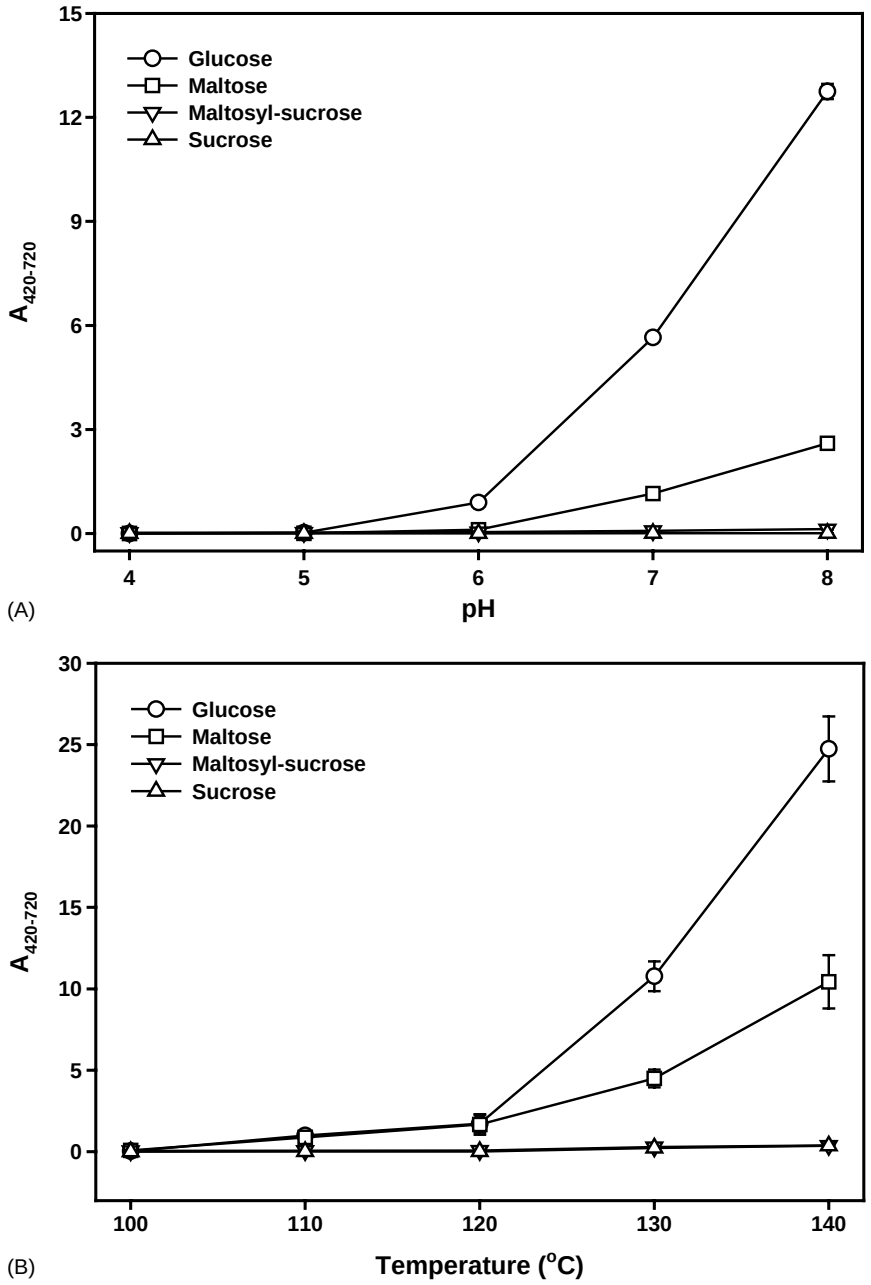

Fig. 7. Colorability of maltosyl-sucrose by the Maillard reaction (A) and heating (B).

by synthesizing extracellular and water-insoluble glucans from sucrose by GTase and by releasing acids from various fermentable sugars. The inhibitory effect of maltosyl-sucrose on mutansucrase activity in the presence of sucrose was tested. When 34 mM of sucrose and 34 mM of maltosyl-sucrose were used as substrates, the enzyme activity was inhibited by 29%, compared to that observed with sucrose (Table 2).

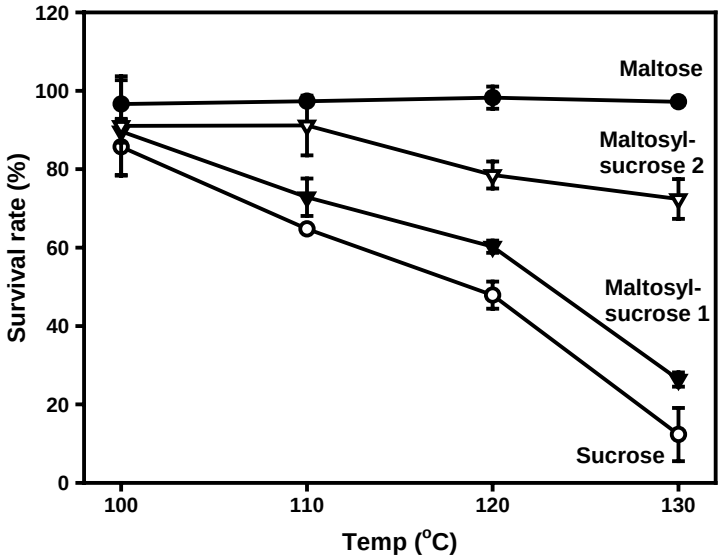

Fig. 8. Stability of maltosyl-sucrose during heating at pH 4.

When 50 mM each of sucrose and maltosyl-sucrose were mixed, the amount of insoluble glucan produced by GTase from *S. sobrinus* was reduced by about 50%, compared to that formed by sucrose alone (Table 2). This phenomenon is similar to those observed with maltose, glucose, fructose, and coupling sugar [7]. In the presence of isomaltosylfructoside, a potent inhibitor of glucan synthesis, a large proportion of low-molecular-weight glucan and a series of nonreducing oligosaccharides are produced [21]. Thus, isomaltosylfructoside acts as an alternative acceptor and serves as an inhibitor to lessen the formation of insoluble glucan. In the case of maltosyl-sucrose, several oligosaccharides were also produced (data not shown), and it was involved in an acceptor reaction to inhibit the synthesis of insoluble glucan.

The rate of fermentation of maltosyl-sucrose was also examined. Fermentation of sugars by oral bacteria produces acid, which has an adverse effect on tooth enamel below a critical pH of 5.7. When 10 mM of sucrose was supplied to the test system of acid fermentation, the pH of the reaction mixture dropped linearly for 20 h of incubation (pH 5.1) and ultimately reached a minimum level of pH 4.0. Maltosyl-sucrose was very easily fermented, but its final pH was slightly higher than that of sucrose.

4. Conclusion

Sucrose was successfully modified by transglycosylation of BSMA to produce various sucrose transfer products. Major transfer products were determined to be 6^{G}-α-maltosyl-sucrose (maltosyl-sucrose **1**) and 6^{F}-α-maltosyl-sucrose (maltosyl-sucrose **2**), in which a maltose binds to the glucose or the fructose moiety

Table 2
Effect of maltosyl-sucrose on inhibition and glucan synthesis of mutansucrase

	Relative activity	Relative ratio of insoluble glucan synthesis[a]	pH after 60 h incubation
Sucrose	100	1	4.0
Sucrose + maltosyl-sucrose	71	0.5	4.6

[a] Relative ratio of insoluble glucan synthesis was calculated by comparing the optical densities at 550 nm. The optical density observed with sucrose was used as a control.

of sucrose by an α-(1,6)-glycosidic linkage. Physicochemical and physiological analyses revealed that these products underwent some significant changes such as decreased cariogenicity.

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