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INHIBITION BY MALTOSE, ISOMALTOSE, AND NIGEROSE OF THE SYNTHESIS OF HIGH-MOLECULAR-WEIGHT D-GLUCANS BY THE D-GLUCOSYLTRANSFERASES OF *Streptococcus sobrinus*

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ABSTRACT

Two D-glucosyltransferases are produced by *Streptococcus sobrinus* C211. One (GTF-S) catalyzes the conversion of sucrose into soluble α-(1→6)-linked α-(1→3)-branched D-glucans, and the other (GTF-I), of sucrose into α-(1→3)-linked α-(1→6)-branched D-glucans. These enzymes were studied by using maltose, isomaltose, and nigerose as inhibitors. Maltose and isomaltose were found to be competitive inhibitors of GTF-S, whereas nigerose has no effect on GTF-S activity. The K_i values for maltose and isomaltose were determined to be 11 and 15mM, respectively. Maltose, isomaltose, and nigerose competitively inhibit GTF-I. The K_i values for these inhibitors were found to be ~0.8, 2.5, and 15mM, respectively. The inhibitory properties of each disaccharide are interpreted in terms of conformational comparisons with sucrose.

INTRODUCTION

The "mutans" streptococci, which include *Streptococcus mutans* and *S. sobrinus*, have been identified as the primary causative agents of dental caries. The bacteria are found as part of the normal microbiota in the oral cavity. Sucrose is utilized by an extracellular enzyme produced by *S. mutans* or *S. sobrinus* to form polysaccharides that mediate attachment of the bacteria to teeth or hard, smooth surfaces, and to other organisms. The extracellular polysaccharides produced by mutans streptococci are synthesized by D-glucosyltransferases (GTF), using sucrose as substrate. These D-glucosyltransferases elaborate two products: one is a water-soluble polymer of D-glucose linked in α-(1→6) fashion and possessing short α-

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(1→3)-linked D-glucose branches. The other is a water-insoluble D-glucan having an α-(1→3)-linked backbone, with longer α-(1→6)-linked chains as branches[1-6]. The enzymes responsible for these polymers are referred to as dextransucrase (GTF-S; EC 2.4.1.4) and mutansucrase (GTF-I; EC 2.4.1.5.), respectively. Two reviews described the role of the D-glucosyltransferases as virulence factors in oral streptococci[7,8].

It is known that GTF can catalyze four types of reaction[9-11]. One is sucrose cleavage, which results in free D-fructose and an enzyme-bound D-glucosyl group. A second is the transfer of the enzyme-bound D-glucosyl group from sucrose to an acceptor molecule; water can serve as an acceptor, thereby completing the hydrolysis of sucrose. Another is polymer formation in the absence of a non-D-glucose acceptor, whereby D-glucose units are linked by addition at the reducing terminus of the growing dextran chain. The last reaction is that of D-fructose exchange, where free D-fructose is able to exchange with the D-fructosyl group of sucrose.

There have been several studies on the inhibition of GTF activities. Competitive inhibitors usually have close structural similarities to sucrose or to acceptor molecules (D-glucans). In the present study, the ability of maltose (4-*O*-α-D-glucopyranosyl-D-glucopyranose), isomaltose (6-*O*-α-D-glucopyranosyl-D-glucopyranose), and nigerose (3-*O*-α-D-glucopyranosyl-D-glucopyranose) to act as inhibitors of GTF preparations was examined. The results showed that maltose and isomaltose are competitive inhibitors of both GTF-S and GTF-I. In contrast, nigerose is a non-inhibitor of GTF-S, but a competitive inhibitor of GTF-I.

EXPERIMENTAL

Bacterial cells and culture conditions. — *Streptococcus sobrinus* C211, a GTF hyperproducing strain, was used for the production[12] of GTF. The bacteria were inoculated into brain–heart infusion-broth (BBL, Cockeysville, MD) and incubated in a 5–10% CO_2 atmosphere at 37°. The cells were then added to Terleckyi and coworkers[13] chemically defined medium containing 2% of D-glucose. The cells were grown overnight under the conditions just described. Bacterial stocks were maintained on Mitis Salivarius agar (Difco, Detroit, MI) and as freeze-dried preparations.

Enzyme isolation and purification. — D-Glucosyltransferase was prepared by precipitation with 60% saturated ammonium sulfate of supernatant liquors of overnight cultures. Precipitated material was collected by centrifugation, dissolved in distilled water, and dialyzed against distilled water and finally against 50mM sodium acetate buffer, pH 5.5. The dialyzate was concentrated, and the concentrate applied to a column[14] of Bio-Gel A-1.5m that was then[14] eluted with 50mM sodium acetate buffer, pH 5.5. Fractions exhibiting GTF-S or GTF-I activity were pooled separately and concentrated.

Determination of GTF activity. — Production of D-glucan from sucrose by

GTF-S was determined by a modification of the procedure of Germaine *et al.*[15]. In a final volume of 450 μL, the reaction mixture contained substrate (2.5 μCi of [U-^{14}C]sucrose, and unlabeled sucrose as needed), 25 μg of dextran T-10 (Pharmacia, Piscataway, NJ), 70 μg of sodium fluoride, and GTF-S in 50mM sodium acetate buffer, pH 5.5. Samples (50 μL) were each spotted onto Whatman 3MM filter-paper disks, and the disks were immediately immersed in absolute methanol. After 15 min, the disks were washed twice with methanol, and dried, and the radioactivity was determined in a scintillation counter.

D-Glucan-producing activity by GTF-I was determined by a modification of the turbidimetric assay of Fukushima *et al.*[16]. A 1-mL reaction mixture contained the following: sucrose, 62 μg of dextran, 155 μg of sodium fluoride, and GTF-I in 50mM sodium acetate buffer, pH 5.5. The increase in absorbance was monitored at 340 nm for 90 min. Activity of the enzyme is reported as the ΔA_{340}/min. When inhibitors were used, they were allowed to preincubate with the enzyme for 15 min prior to addition to the reaction substrate.

Chemicals. — Maltose was obtained from Pfanstiehl Laboratories, Incorporated, Waukegan, IL. Isomaltose and nigerose were obtained from Sigma Chemical, St. Louis, MO. Radioactive sucrose was obtained from ICN Chemicals, Irving, CA.

RESULTS AND DISCUSSION

Maltose and isomaltose had been shown to inhibit the synthesis of insoluble and soluble D-glucan from sucrose by GTF preparations[17–19]. Robyt and Eklund[17] found that these disaccharides and nigerose can act as acceptors for the D-glucosyl groups generated in GTF (*Leuconostoc mesenteroides* B-512F) catalysis, thereby terminating the polymerization of D-glucose. In GTF reactions where an acceptor ("primer") is employed, linear sequences of α-(1→6)-linked D-glucans with some α-(1→3) branching perform as the best stimulators of GTF activity. Linear sequences of α-(1→3)-linked D-glucan have no effect on GTF activity[19]. The active site(s) of GTF probably recognize structural features of these primer D-glucans that promote optimal syntheses of α-(1→6) and α-(1→3) bonds. Yet smaller molecules possessing the same basic structural features inhibit D-glucan synthesis. A needed extension of the above studies is the characterization of the inhibitory effects of disaccharides containing α-(1→6) and α-(1→3) linkages on individual GTF-I and GTF-S activities.

Lineweaver–Burk plots of typical experiments using a 5mM inhibitor concentration and various sucrose concentrations show that maltose, isomaltose, and nigerose are competitive inhibitors of the synthesis of insoluble D-glucan (see Fig. 1). Maltose was the most effective inhibitor of GTF-I, followed by isomaltose, and then nigerose. Dixon plots (not shown) to determine kinetic constants* for each inhibitor revealed K_i values of 0.8, 2.5, and 15mM, respectively. With the

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**Editor's note:* Because there are only two lines in each Lineweaver–Burk plot, this determination involves the assumption that the slopes of the lines are a linear function of the concentration of the inhibitor.

apparent K_m of sucrose for GTF-I at 3.7mM, maltose appears to bind the active site for GTF-I more than four-fold better than sucrose or the D-glucosyl group of sucrose.

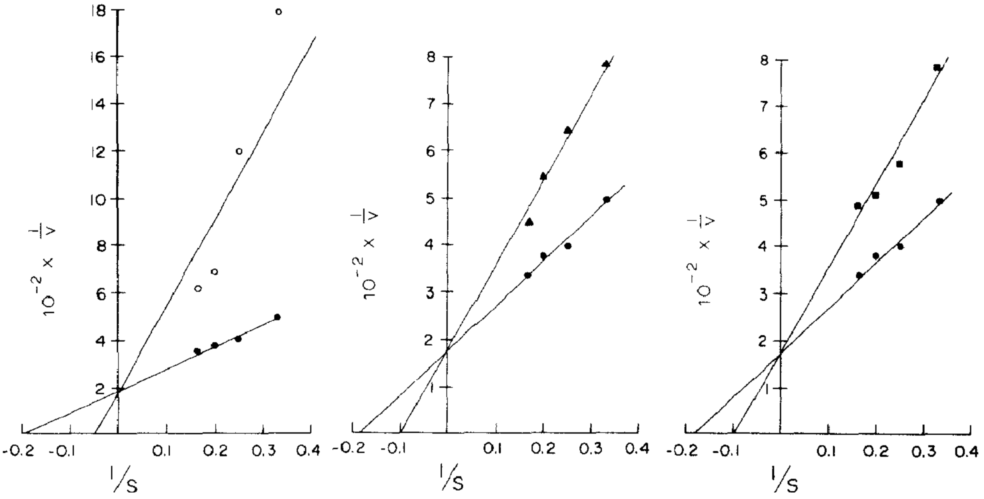

Fig. 1. Lineweaver–Burk plots showing the inhibition of GFT-I with maltose (○), isomaltose (▲), and nigerose (■). Reaction mixtures contained sucrose and dextran in a sodium acetate buffer, pH 5.5. GTF-I control reactions (●) contained no inhibitors. Inhibitors (5mM) were preincubated with GTF-I for 15 min before addition to reaction mixture. The reaction was allowed to proceed, and the change in absorbance at 340 nm was recorded. V = Δ absorbance/min; S = mM.

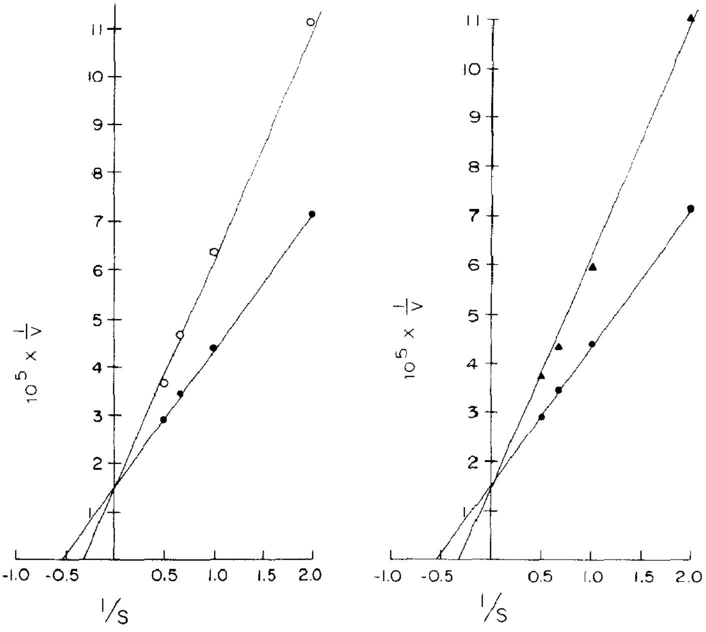

Fig. 2. Lineweaver–Burk plots of GTF-S with maltose (○) and isomaltose (▲). Reaction mixtures contained labeled sucrose and dextran in a sodium acetate buffer at pH 5.5. Inhibitors (5mM) were preincubated with GTF-S for 15 min prior to addition to the reaction mixture. GTF-S control reactions (●) contained no inhibitors. The reaction was allowed to proceed for 15 min. V = c/m; S = mM.

Lineweaver–Burk plots of typical experiments describing the kinetics of inhibition of GTF-S by maltose and isomaltose are shown in Fig. 2. Inhibitor concentration was 5mM. Maltose and isomaltose were shown to be competitive inhibitors of soluble D-glucan synthesis. When nigerose was used as an inhibitor, no inhibition of soluble D-glucan synthesis occurred, even at a concentration of 20mM. Kinetic constants (K_i) for maltose and isomaltose with GTF-S were 11 and 15mM, respectively.

The results with nigerose and GTF-S were unexpected, because Robyt and Eklund[17] had reported that nigerose can act as an acceptor of D-glucosyl units from the dextransucrase of *Leuconostoc mesenteroides* B-512F. They suggested that nigerose terminates D-glucan synthesis in α-(1→6) linkages. Nisizawa *et al.*[20] have shown that maltose and isomaltose or isomaltosyl D-fructoside are able to act as "alternative acceptors" for the D-glucosyl groups generated by GTF and sucrose. The oligosaccharides produced in these reactions include panose, or D-glucans terminated by isomaltose or nigerose at the reducing end.

The results describing inhibition of GTF-S and GTF-I (see Figs. 1 and 2) may not actually indicate suppression of enzyme activity, but could reflect termination reactions resulting in products having lower molecular weights. Sato *et al.*[21] observed that various D-glucose-containing oligosaccharides cause the GTFs of *S. sobrinus* to produce low-molecular-weight D-glucans from sucrose. In addition, Robyt and Eklund[17] found that maltose decreases the degree of polymerization of D-glucans synthesized by *L. mesenteroides* B-512F GTF-S. Nigerose and isomaltose were found much less effective in lessening the degree of polymerization of the D-glucans. Robyt and Martin[22] showed that the D-glucosyl group of sucrose is transferred to the reducing end of the D-glucan acceptor, and they proposed a mechanism for D-glucan synthesis from GTFs in which such saccharides as maltose, isomaltose, or nigerose can act as acceptors, thereby terminating the production of high-molecular-weight D-glucans without necessarily diminishing the catalysis of sucrose.

The key stereochemical differences between maltose, isomaltose, and nigerose (all of which have D-glucose units in the $^4C_1$ conformation) are those imparted by the structural position of the glycosidic linkage and the favored rotational isomerism about that linkage. The favored conformations of maltose, isomaltose (as approximated by melibiose), and nigerose are shown in Figs. 3A, B, and C, respectively. The key structural features and the source of structural data for each disaccharide are outlined next.

In general, the solid-sate structure of a disaccharide is a good representation of its structure in aqueous solution[23]. X-Ray crystallographic data[23] for β-maltose monohydrate showed that the torsional angle described by atoms O-5–C-1–O-1–C-14 is +122°, and that that described by atoms C-1–O-1–C-14–C-13 is +133° (numbering as shown in the Figures). These angles are close to those determined for anhydrous maltose. The minimum-energy conformation of maltose has been estimated by HSEA calculations, and the conformation in aqueous solution has been confirmed by two n.m.r. techniques[24–26]. Solvent changes induce con-

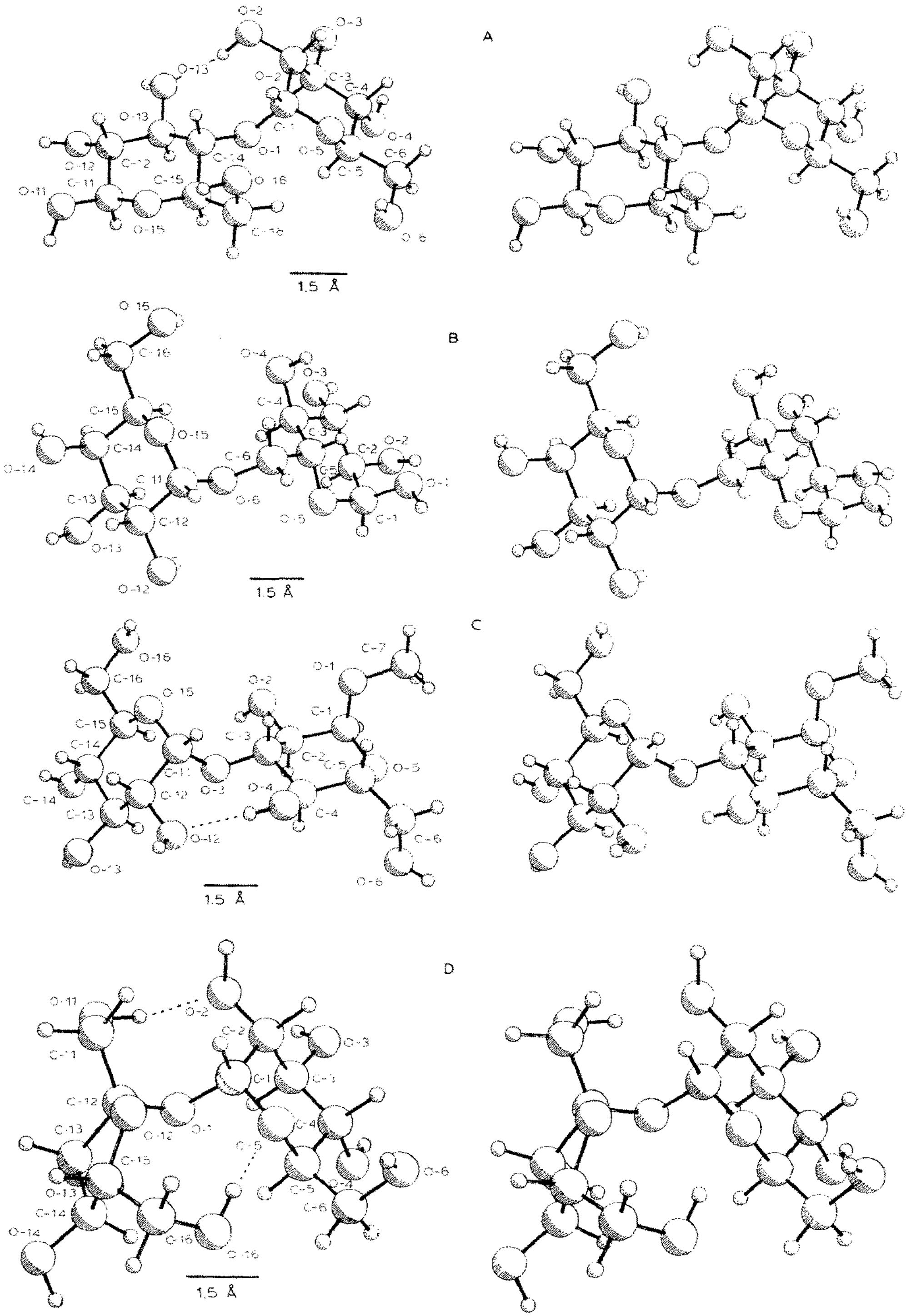

Fig. 3. Stereoviews of the solid-state structures of (A) maltose, (B) melibiose, (C) methyl α-nigeroside, and (D) sucrose.

formational changes in maltose, but the two major contributing conformations in water are still very similar to that shown in Fig. 3A. The results indicated that the conformation in solution is similar to the solid-state structure depicted in Fig. 3A.

For isomaltose or a simple derivative, no X-ray crystallographic structure has yet been reported. However, the likely solid-state structure can be inferred from that[27] of α-melibiose (6-*O*-α-D-galactopyranosyl-D-glucopyranose), which differs only in the configuration of C-14. The key torsional angles and their values are as follows: O-15–C-11–O-6–C-6 = +76°, C-11–O-6–C-6–C-5 = −174°, and O-6–C-6–C-5–O-5 = −64°. The measurement of $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$ by Ohrui *et al.*[28] confirmed that the *g,g* orientation for the glycosidic linkage (see Fig. 3B) is the major one in aqueous solutions.

The X-ray crystallographic data for the methyl α-glycoside of nigerose (see Fig. 3C) shows the following torsional angles: O-15–C-11–O-3–C-3 = +104° and C-11–O-3–C-3–C-4 = +104°. We have performed HSEA calculations on nigerose to estimate the minimum-energy conformation, and the results are consistent with the solid-state structure[29].

Thus, the three molecules present unique structures to the GTF enzymes. Maltose possesses hydrophobic and hydrophilic "edges". These are described by the atom groupings of C-16, O-15, C-6, and O-5, and OH-4, OH-3, OH-2, OH-13, and OH-12, respectively. However, the hydrophilic edge possesses a relatively hydrophobic region resulting from the intramolecular hydrogen-bond between OH-2 and OH-13. Interestingly, these general features are similar to those of sucrose[30], the substrate of the GTFs (see Fig. 3D). Comparison of Fig. 3A and 3D reveals the similarities of the two molecules in the regions described. For sucrose, the hydrophobic and hydrophilic atom groupings are C-16, O-16, O-5, and C-6, and OH-4, OH-3, OH-2, OH-11, and O-13, respectively. With sucrose, as well, the intramolecular hydrogen-bond between OH-2 and OH-13, which is maintained in solution[31,32], imparts a hydrophobic region to the hydrophilic edge. Isomaltose has a much more flexible D-glucosidic linkage, and, as a result, possesses general structural features similar to those of methyl α-D-glucopyranoside, another weak competitive inhibitor of GTF. In contrast, nigerose possesses relatively hydrophobic surfaces: C-16, O-15 (which hinder OH-2), C-6, O-5, and the intramolecular hydrogen-bond between OH-4 and OH-12. The work of Binder and Robyt[33] with hydrophobic derivatives of sucrose indicated that GTF-I possesses the more hydrophobic and less selective binding sites. Thus, the present results are generally consistent with Binder and Robyt's findings[33].

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