Carbohydrate Research, 147 (1986) 135–144
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

DIFFERENCE IN MODE OF INHIBITION BETWEEN α-D-XYLOSYL β-D-FRUCTOSIDE AND α-ISOMALTOSYL β-D-FRUCTOSIDE IN SYNTHESIS OF GLUCAN BY *Streptococcus mutans* D-GLUCOSYLTRANSFERASE*

TOSIKI NISIZAWA, KANOU TAKEUCHI**, SUSUMU IMAI,

Department of Dental Research, National Institute of Health, 2-10-35, Kamiosaki, Shinagawa-ku, Tokyo 141 (Japan)

SUMIO KITAHATA, AND SHIGETAKA OKADA

Department of Biochemistry, Osaka Municipal Technical Research Institute, 2-1-1, Oogimachi, Kita-ku, Osaka 530 (Japan)

(Received March 18th, 1985; accepted for publication in revised form, August 8th, 1985)

ABSTRACT

Both α-isomaltosyl β-D-fructoside and α-D-xylosyl β-D-fructoside show strong inhibition of the synthesis of water-insoluble and water-soluble D-glucans from sucrose by a partially purified preparation of a D-glucosyltransferase (GTase) from *Streptococcus mutans* 6715; however, the inhibitory modes differ substantially. In the presence of α-isomaltosyl β-D-fructoside, the production of reducing sugars and the consumption of sucrose are remarkably enhanced, compared with a control of sucrose alone. Under these conditions, a large proportion of low-molecular-weight glycan (lmwg) and a series of nonreducing oligosaccharides (both containing D-fructosyl groups or residues) are produced. In contrast, in the presence of α-D-xylosyl β-D-fructoside, the production of reducing sugars and the sucrose consumption are strikingly suppressed, and no lmwg or oligosaccharides are produced. Thus, it may be concluded that α-isomaltosyl β-D-fructoside acts as an alternative acceptor for the D-glucosyl and/or D-glucanosyl transfer reactions of the enzyme, and serves to lessen the formation of insoluble and soluble D-glucan, although it stimulates the transferring activity of the enzyme. On the other hand, α-D-xylosyl β-D-fructoside competitively inhibits the sucrose-splitting activity of the enzyme as an analog to sucrose, and thereby diminishes the synthesis of D-glucan.

INTRODUCTION

Sucrose 6-glucosyltransferase (GTase: EC 2.4.1.5) of *Streptococcus mutans* is one of the main, causative factors involved in the occurrence of dental caries as the synthesis of water-insoluble D-glucan from sucrose by the enzyme causes bacterial

*Supported, in part, by Grant-in-Aid for Scientific Research 59570878 (1984).
**Present address: Hayashibara Biochemical Laboratories Incorporated, 1-2-3, Shimoishii, Okayama 700, Japan.

0008-6215/86/$ 03.50

adherence to, and plaque accumulation on, tooth surface[1-3]. This sucrose-dependent, insoluble D-glucan synthesis by streptococcal GTase is remarkably inhibited by D-fructose, maltose, isomaltose, and many other saccharides, including methyl sugars and amino sugars[4-10]. Recently, we reported[11] that α-isomaltosyl β-D-fructoside and α-D-xylosyl β-D-fructoside also strongly inhibit the synthesis of D-glucan from sucrose by a crude preparation of the extracellular GTase of *S. mutans* 6715.

We now describe the inhibitory action of α-isomaltosyl β-D-fructoside and α-D-xylosyl β-D-fructoside against the *in vitro* synthesis of D-glucan by a partially purified GTase preparation, and shall discuss the difference between the modes of their inhibition.

MATERIALS AND METHODS

Sugars. — α-D-Xylopyranosyl β-D-fructofuranoside [α-D-Xyl*p* β-D-Fru*f*] was synthesized by the method of Avigad *et al.*[12], using purified levansucrase of *Bacillus subtilis* var. *saccharolyticus* as described previously[11]. Briefly, an aqueous solution containing 2% of sucrose and 10% of D-xylose was incubated with the enzyme for 48 h at 40°. α-D-Xylosyl β-D-fructoside was isolated from the mixture by charcoal-column chromatography, with 2:23 (v/v) ethanol–water as the eluate. The yield was 14.3%. α-Isomaltosyl β-D-fructoside [*O*-α-D-Glc*p*-(1→6)-α-D-Glc*p* β-D-Fru*f*] was synthesized by essentially the same method, except that isomaltose was used as the acceptor. The trisaccharide was isolated by charcoal-column chromatography with 3:1 (v/v) ethanol–water. The yield was 18%. The synthetic sugars, identified by optical rotation and by enzymic hydrolysis using yeast β-D-fructofuranosidase (Boehringer–Mannheim–Yamanouchi, Tokyo) as described previously[11], each gave a single spot on paper chromatograms.

Preparation of glucosyltransferase (GTase). — The partially purified GTase preparation was obtained from a brain–heart infusion–broth (Difco Laboratories, Detroit, MI) culture of *Streptococcus mutans* strain 6715 by a modification of the procedure[13] of Smith *et al.* A crude GTase fraction was prepared by precipitating it from the culture supernatant liquor with a 50% saturation of ammonium sulfate, followed by dialysis against 0.05M sodium phosphate buffer, pH 6.8, containing 0.02% of sodium azide, and the enzyme was incubated with 10% sucrose for 16 h at 37°. The product, insoluble D-glucan–GTase complex, was washed twice with the aforementioned buffer and agitated for 24 h at 4° in 10M lithium chloride solution to remove the weakly bound contaminating proteins. The GTase was not released from the D-glucan by this treatment. The lithium chloride was removed from the complex by washing with the buffer, and the GTase was dissociated from the D-glucan with 6M guanidine hydrochloride for 1 h at room temperature. The guanidine solution was then extensively dialyzed against 0.01M sodium phosphate buffer, pH 6.8, containing 0.02% of sodium azide, and the partially purified preparation was used as the enzyme. One unit of GTase is defined as that amount

which will liberate 1.0 μmol of reducing-sugar equivalent, as D-glucose, per min under the standard assay conditions; 1 mL of the GTase preparation contained 50 μg of proteins and 0.02 unit of GTase. The specific activity (units/mg of proteins) of the enzyme preparation relative to that of the crude GTase fraction was 451.9 and the yield of the enzyme was 41.5%. This GTase preparation did not contain any D-fructosyltransferase and could not release any detectable reducing sugar from dextran T2000 (substrates for dextranase), inulin (for inulinase), raffinose (for invertase and D-fructosyltransferase), α-isomaltosyl β-D-fructoside, or α-D-xylosyl β-D-fructoside even after incubation for 20 h at 37° (data not shown), but it still contained two active GTase proteins synthesizing insoluble D-glucan and soluble D-glucan[13–15].

Enzyme assay. — The reaction mixture contained 50mM sodium phosphate buffer (pH 6.8), 5mM sucrose, 3mM sodium azide, and 0.004 unit of the GTase in a final volume of 4 mL. After incubation for an appropriate period at 37°, in the presence or absence of maltose, α-D-xylosyl β-D-fructoside, or α-isomaltosyl β-D-fructoside, in a small glass test-tube, insoluble D-glucan (IG) produced in the reaction mixture was separated by centrifugation at 7,200*g* for 20 min. The precipitated IG and the IG that adhered to the test tube were washed twice with the buffer, and dissolved in 0.5M sodium hydroxide (1 mL). To the supernatant liquor from the reaction mixture was added 9:11 ethanol–water, and the mixture was kept for 18 h at 4°, to precipitate[16] soluble D-glucan (SG). Low-molecular-weight D-glycan (lmwg) in the supernatant liquor from the last ethanol fractionation was precipitated by addition of ethanol up to 75%, and the mixture was kept for 18 h at 4°. Sugars still dissolved in the supernatant liquor from this ethanol fractionation were analyzed by paper chromatography, or were further fractionated into mono-, di-, and oligo-saccharides by charcoal-column chromatography, or both.

Determination of the values of K_m and K_i. — The standard reaction-mixtures containing various concentrations (2.5–50mM) of sucrose were incubated for 120 min at 37° with or without 10mM α-D-xylosyl β-D-fructoside. The reaction mixtures were then assayed for reducing power. The velocity of the reaction was expressed as the amounts (μg) of reducing sugars (as D-glucose) per mL of the reaction mixture, and the data were plotted by the method of Lineweaver and Burk.

Analytical methods. — Gas–liquid chromatography was conducted in a Shimadzu gas Chromatograph GC-7AG. Separations were made at a nitrogen flow-rate of 40 mL per min on a column (3 mm by 250 cm) containing 3% (w/w) of ECNSS-M on Gaschrom Q (100–120 mesh), the temperature in the oven being increased from 230 to 270° in 15 min, and the sample injected at 300°.

Charcoal-column chromatography was performed in a column (6 mm by 8 cm) containing activated charcoal (4 g). The column was eluted stepwise with 0:100, 1:19, 1:9, and 1:4 ethanol–water.

Paper chromatography was carried out as described previously[16]. Reducing

sugars on the chromatograms were detected with the acetone–silver nitrate reagent, and ketoses with the phloroglucinol reagent[17].

The amounts of IG, SG, lmwg, and oligosaccharides were determined colorimetrically by the phenol–sulfuric acid method[18]. Each sugar in the mono- and di-saccharide fractions was identified, and simultaneously quantified, as the trimethylsilyl derivatives[19] or alditol acetate derivative[20] by gas–liquid chromatography. The amounts of D-glucose and fructose were also respectively determined by the D-glucose oxidase method (Blood Sugar GOD Perid Test: Boehringer–Mannheim) and the anthrone–sulfuric acid method[21] at 40°. Reducing sugar was analyzed by the colorimetric method of Somogyi–Nelson[22]. Protein was determined by the method of Lowry *et al.*[23].

RESULTS

The partially purified GTase preparation was incubated with 5mM sucrose (1.7 mg/mL) for 16 h at 37° in the absence or presence of various concentrations of α-D-xylosyl β-D-fructoside or maltose. The amounts of products produced in the reaction mixture were determined, and these are plotted in Fig. 1. During the 16-h incubation, ~2.8 mg of sucrose was consumed in the absence of the xylosyl

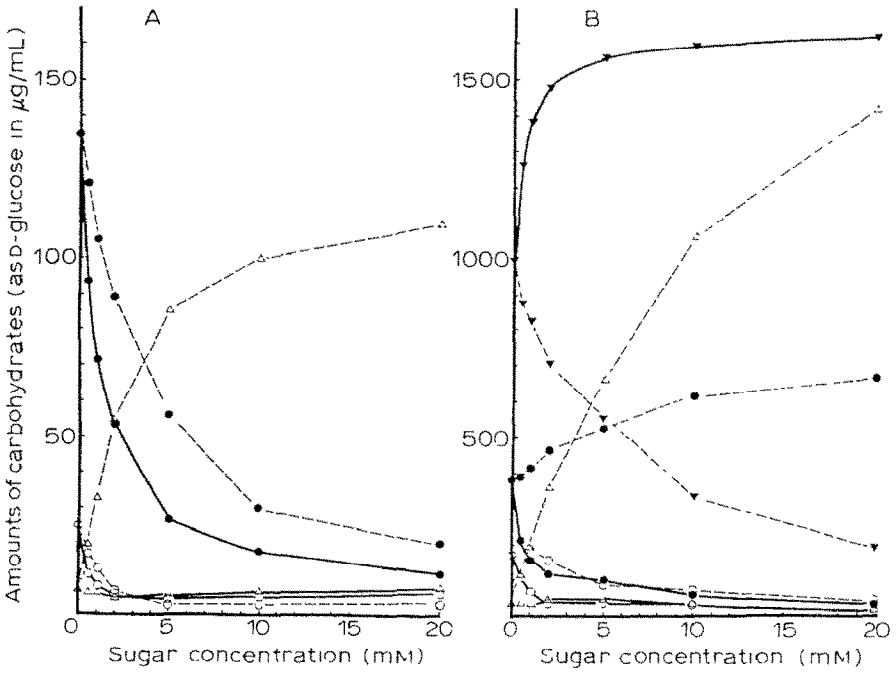

Fig. 1. Effects of various concentrations of maltose (----) or α-D-xylosyl β-D-fructoside (——) on the amounts of products from the GTase–sucrose reactions. A: ●, IG (insoluble D-glucan); ○, SG (soluble D-glucan); and △, lmwg (low-molecular-weight glycan). B: ●, D-fructose, ○, D-glucose; △, oligosaccharides; and ▼, residual sucrose

fructoside or maltose (control), and an almost equivalent amount of products was obtained from the sucrose in the reaction mixture (4 mL): ~540 μg of IG, 100 μg of SG, 720 μg of D-glucose and 1,480 μg of D-fructose. Almost negligible amounts of lmwg and oligosaccharides were produced, and ~4.0 mg of residual sucrose was recovered from the mixture.

The incubation of the enzyme preparation with sucrose and maltose resulted in an increase in the amounts of lmwg, oligosaccharides, D-fructose and consumed sucrose in the reaction mixture with increasing concentrations of maltose, but the amounts of IG, SG, and D-glucose decreased. A decrease in the amounts of IG, SG, and glucose was also observed on addition of xylosyl fructoside instead of maltose. However, in this case, the production of D-fructose and the consumption of sucrose in the reaction mixture fell with its increase in concentration (in contrast to maltose), and no significant amount of lmwg and oligosaccharides was detected.

Fig. 2 shows the time courses of the amounts of IG, SG, lmwg, and residual sucrose in the reaction mixture. The production of IG and SG was remarkably diminished by the presence of maltose, isomaltosyl fructoside, or xylosyl fructoside,

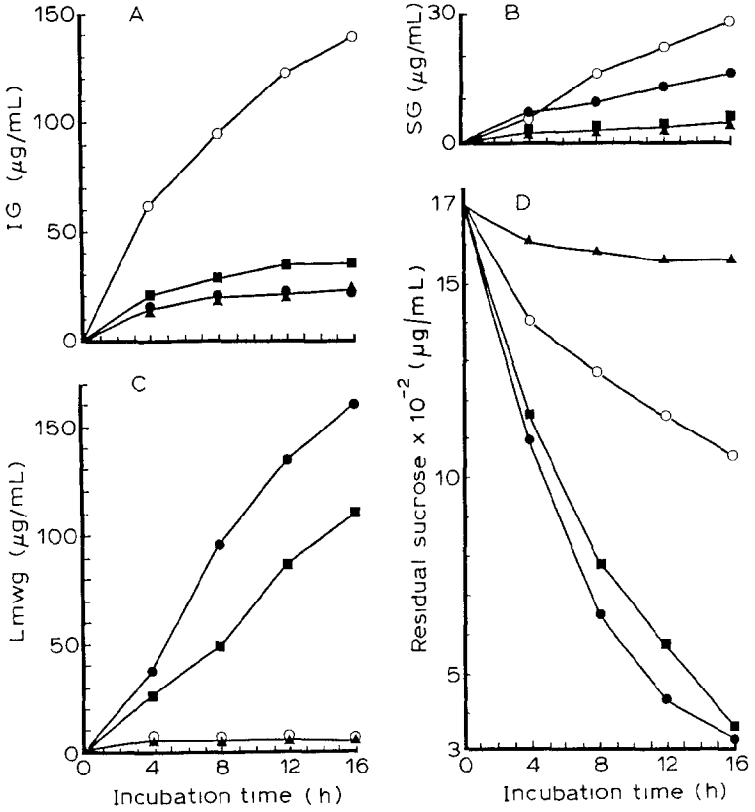

Fig. 2. Time courses of the production of IG (A), SG (B), or lmwg (C) from 5mM sucrose by the GTase preparation, and of amount of residual sucrose (D), in the absence (○) or presence of 10mM maltose (■), α-isomaltosyl β-D-fructoside (●) or α-D-xylosyl β-D-fructoside (▲).

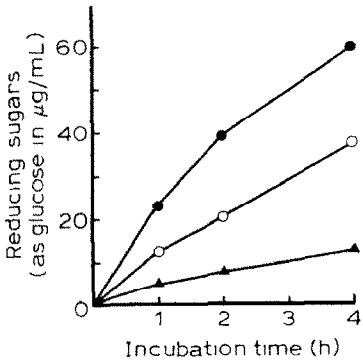

Fig. 3. Time course of the production of reducing sugars from 5mM sucrose by the GTase preparation in the absence (○) and presence of 10mM α-isomaltosyl β-D-fructoside (●) or α-D-xylosyl β-D-fructoside (▲).

whereas the amounts of lmwg increased with time when maltose or isomaltosyl fructoside was added. Regardless of the presence or absence of xylosyl fructoside, the lmwg was not significantly produced from sucrose. The lmwg formed in the presence of isomaltosyl fructoside contained a detectable amount of fructosyl residues (examination by the anthrone–sulfuric acid method at 40°), but that produced in the presence of maltose did not (data not shown). The consumption of

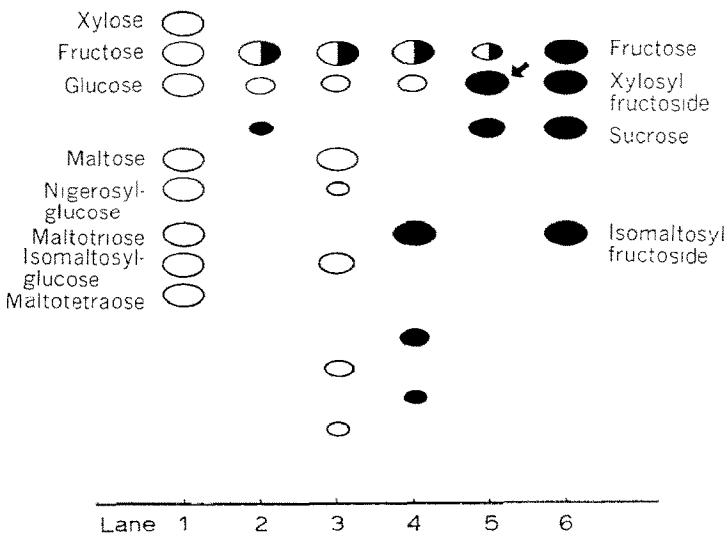

Fig. 4. Diagrammatic representation of the paper chromatogram of oligosaccharides in the 75% ethanol supernatant liquor from the mixture resulting from 16-h incubation with sucrose alone (lane 2), sucrose and maltose (3), sucrose and α-isomaltosyl β-D-fructoside (4), or sucrose and α-D-xylosyl β-D-fructoside (5). Lanes 1 and 6 are those of authentic standards. Sugars were detected with the silver nitrate reagent (○) or the phloroglucinol reagent (●). ◑: sugars visibilized with both reagents. ◂: spot containing traces of glucose visibilized with the silver nitrate reagent.

sucrose was accelerated in the presence of maltose or isomaltosyl fructoside as compared with the control. In contrast, only a small amount of sucrose was consumed in the presence of xylosyl fructoside. In addition, it is clear (see Fig. 3) that the production of reducing sugar from sucrose by the GTase preparation in the reaction mixture was also accelerated by the presence of isomaltosyl fructoside, but suppressed by xylosyl fructoside.

In Fig. 4, the paper chromatograms of the sugars in the supernatant liquor from the 75%-ethanol fraction obtained from the 16-h incubation mixture are shown diagrammatically. Almost no oligosaccharide was found in the reaction mixture when sucrose alone (control) is, or sucrose and xylosyl fructoside are, present. Whereas, in the presence of maltose plus sucrose, several reducing oligosaccharides, such as a considerable amount of panose (isomaltosyl-D-glucose) [*O*-α-D-Glc*p*-(1→6)-*O*-α-D-Glc*p*-(1→4)-D-Glc] and a trace of nigerosyl-D-glucose [*O*-α-D-Glc*p*-(1→3)-*O*-α-D-Glc*p*-(1→4)-D-Glc], are produced in the reaction mixture, judging from their mobilities. In the presence of isomaltosyl fructoside instead of maltose, some oligosaccharides were also detected; however, these were not colored by the silver nitrate reagent, but only by the phloroglucinol reagent, suggesting the formation of nonreducing saccharides containing fructose.

Various increasing amounts of sucrose were incubated with the GTase preparation for 120 min at 37° in the absence or presence of 10mM xylosyl fructoside, in order to measure the K_m and K_i values. The reaction mixtures were then assayed for reducing power. The results and Lineweaver–Burk plot of the data are

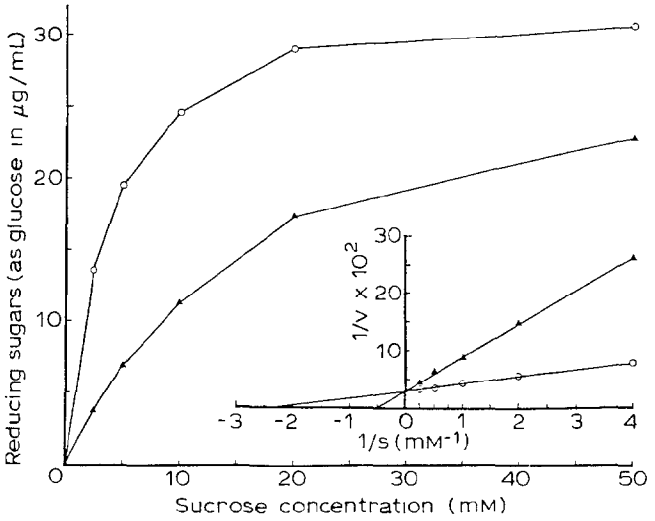

Fig. 5. Effect of substrate concentration on reaction velocity of the GTase in the absence (○) or presence (▲) of 10mM α-D-xylosyl β-D-fructoside. Inset: Lineweaver–Burk plot of the data. Vertical line represents the reciprocal of the maximum velocity of the enzyme reactions, expressed as the total amount of reducing sugars released from sucrose. Horizontal line represents the reciprocal of the sucrose concentration.

shown in Fig. 5. The production of reducing sugars by the enzyme preparation was competitively inhibited by xylosyl fructoside, and the apparent K_m and K_i values were calculated to be 4.4 and 2.8mM, respectively.

DISCUSSION

It is well documented that, when used at relatively high concentrations, such sugars as maltose, isomaltose, maltotriose, and isomaltotriose can effectively inhibit the synthesis of insoluble D-glucan from sucrose by streptococcal GTase[4-8,11]. These sugars also act as alternative acceptors for the D-glucosyl groups generated in the GTase–sucrose reaction to change into the corresponding oligosaccharides, in which chain elongation occurs by attachment of the D-glucosyl groups through α-D-(1→6)-glucosidic linkages at the nonreducing end of the acceptors[24-26]. Such monosaccharides as D-glucose, D-fructose, and methyl α-D-glucoside, known to be inhibitors of the synthesis of insoluble D-glucan, also act as alternative acceptors for D-glucosyl or glucanosyl transfer reactions, or both, of the enzyme[9,10]. Thus, Robyt and co-workers[9,27] proposed that the acceptor reactions of such disaccharides as maltose serve to terminate polymerization of D-glucan by displacing the growing D-glucan chain from the active site of the enzyme and thereby lessen the formation of insoluble D-glucan.

In the present work, we used maltose as a reference inhibitor of the synthesis of IG and SG from sucrose by the GTase (see Figs. 1 and 2). The utility of maltose as an acceptor was revealed by the facts that large amounts of lmwg (see Figs. 1 and 2) and several reducing oligosaccharides, such as panose and nigerosyl-D-glucose (see Fig. 4), were produced from sucrose in the presence of maltose, and neither of them contained any detectable amount of fructose. In the absence of maltose (sucrose alone), almost no lmwg and oligosaccharides were produced (see Figs. 1, 2, and 4). Interestingly, a detectable trace of reducing compound, seemingly nigerosyl-D-glucose, was produced from sucrose in the presence of maltose (see Fig. 4). Although definite identification of this compound will be necessary in order to draw a concrete conclusion, the fact suggests that the GTase preparation used may be capable of transferring the D-glucosyl group of sucrose to O-3 of the D-glucosyl group (nonreducing) of maltose, even if it results from minor or mistransfer reactions of the enzymes under the present experimental conditions. With regard to this possibility, Walker[24,28] reported that the oligosaccharides produced from the maltose or isomaltose acceptor-reactions by *S. sanguis* GTase contain no α-D-(1→3)-glucosidic linkages, whereas *S. mutans* OMZ 176 GTase could introduce a D-glucosyl branch with an α-D-(1→3) linkage into isomaltose saccharides containing more than 4 D-glucosyl residues.

Isomaltosyl fructoside, a theoretically appropriate intermediate or acceptor, or both, in dextran synthesis[24,29,30], inhibits the formation of IG and SG by the mode described for maltose, because large amounts of lmwg and nonreducing oligosaccharides (both containing D-fructosyl residues) were produced in the reac-

tion mixture (see Figs. 2 and 3). In addition, the stimulation of sucrose-splitting activity or of D-glucosyl-transferring activity, or both, of the GTase, suggested by the increase in the amounts of D-fructose from sucrose with increasing concentration of maltose (see Fig. 1), was also observed in the presence of isomaltosyl fructoside instead of maltose (see Fig. 3). Thus, it is clear that the consumption of sucrose by the enzyme was remarkably accelerated in the presence of isomaltosyl fructoside, as well as maltose (see Fig. 2).

Xylosyl fructoside also inhibited the syntheses of IG and SG (see Figs. 1 and 2). However the mode of inhibition is considered to differ substantially from that of the other sugars, as the production of lmwg and oligosaccharides from sucrose was almost negligible in this case (see Figs. 1, 2, and 4); this suggests that xylosyl fructoside could not act as an acceptor for the D-glucosyl or D-glucanosyl units, or both, generated in the GTase–sucrose reactions, in contrast to other sugars so far reported to be inhibitors of the synthesis of insoluble D-glucan by GTase. Xylosyl fructoside probably inhibits the sucrose-splitting activity of the enzyme competitively, as an analog of sucrose, and thereby lessens the synthesis of D-glucan, because the generation of D-fructose or other reducing sugars by the enzyme–sucrose reactions, and consumption of sucrose are positively suppressed by the addition of this disaccharide (see Figs. 1–3). The result from the Lineweaver–Burk plot (see Fig. 5) also supports this conclusion, although the K_m and K_i values obtained are only apparent values for the cooperative activities of two kinds of GTase in the enzyme preparation.

Luzio and Mayer[31] reported that GTase catalyzes the hydrolysis of sucrose to D-glucose and D-fructose, a reaction in which water may act as an acceptor for the D-glucosyl-transferring activity of the enzyme. The GTase preparation used herein contained no dextranase, inulinase, invertase, or D-fructosyltransferase activity (see Materials and Methods). Thus, the production of free D-glucose in the reaction mixture (see Fig. 1) may be due to hydrolytic activity of the GTases. For the hydrolysis of sucrose by GTase, according to the insertion mechanism[32], cooperation of the both sucrose-splitting and D-glucosyl-transferring activities to water must be necessary. Xylosyl fructoside may therefore diminish the production of free D-glucose by inhibition of the sucrose-splitting activity of the GTase (see Fig. 1). In contrast, the lessening of the production of free D-glucose observed in the presence of maltose (see Fig. 1) may be caused by the acceptor-reaction of the disaccharide, which probably stimulates the D-glucosyl-transferring activity of the enzyme.

REFERENCES

1 H. W. SCHERP, *Science*, 173 (1971) 1199–1205.
2 R. J. GIBBONS AND J. VAN HOUTE, *Annu. Rev. Med.*, 26 (1975) 121–136.
3 R. J. FITZGERALD, in H. M. STILES, W. J. LOESCHE, AND T. C. O'BRIEN (Eds.), *Microbial Aspects of Dental Caries*, Information Retrieval, Washington, DC, 1976, pp. 840–858.
4 R. J. GIBBONS AND M. NYGAARD, *Arch Oral Biol.*, 13 (1968) 1249–1262.
5 A. M. CHLUDZINSKI, G. R. GERMAINE, AND C. F. SCHACHTELE, *J. Dent. Res., Special Issue C*, 55 (1976) c75–c86.

6 E. NEWBRUN, F. FINZEN, AND M. SHARMA, *Caries Res.*, 11 (1977) 153–159.
7 S. HAMADA AND M. TORII, *J. Gen. Microbiol.*, 116 (1980) 51–59.
8 S. THANIYAVARN, S SINGH, C. M. MAYNARD, K. G. TAYIOR, AND R J. DOYLE, *Carbohydr. Res.*, 96 (1981) 134–137.
9 J. F ROBYT AND T. F. WALSETH, *Carbohydr. Res.*, 61 (1978) 433–445.
10 R. M. MAYER, M. M. MATTHEWS, C. L. FUTERMAN, V. K. PARNAIK, AND S. M. JUNG, *Arch. Biochem. Biophys.*, 208 (1981) 278–287.
11 S. IMAI, K. TAKEUCHI, K SHIBATA, S. YOSHIKAWA, S. KITAHATA, S OKADA, S ARAYA, AND T. NISIZAWA, *J. Dent. Res.*, 63 (1984) 1293–1297.
12 G. AVIGAD, D. S. FEINGOLD, AND S. HESTRIN, *Biochim. Biophys. Acta*, 20 (1956) 129–134.
13 D. J. SMITH, M. A. TAUBMAN, AND J. L. EBERSOLE, *Infect. Immun.*, 23 (1979) 446–452.
14 T. FURUTA, T. NISIZAWA, J CHIBA, AND S. HAMADA, *Infect. Immun.*, 41 (1983) 872–875.
15 T. FURUTA, T KOGA, T. NISIZAWA, N. OKAHASHI, AND S. HAMADA, *J Gen Microbiol.*, 131 (1985) 285–293.
16 T. NISIZAWA, S. IMAI, H. AKADA, M. HINOIDE, AND S. ARAYA, *Arch. Oral Biol.*, 21 (1976) 207–213
17 I. SMITH (Ed), *Chromatographic Techniques Clinical and Biochemical Applications*, Interscience, New York, 1958, p. 168.
18 M. DUBOIS, K. A. GILLES, J K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
19 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Am. Chem. Soc.*, 85 (1963) 2497–2507.
20 H. BJORNDAL, B. LINDBERG AND S SVENSSON, *Acta Chem. Scand.*, 21 (1967) 1801–1804.
21 J. R. HELBERT AND K D. BROWN, *Anal. Chem.*, 27 (1955) 1791–1796.
22 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375–380.
23 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, AND R J. RANDALL, *J. Biol Chem.*, 193 (1951) 265–275.
24 G. J. WALKER, *J. Dent. Res.*, 51 (1972) 409–414.
25 S. M. JUNG AND R. M. MAYER, *Arch. Biochem. Biophys.*, 208 (1981) 288–295.
26 K FUKUI AND T. MORIYAMA, *Microbiol. Immunol.*, 27 (1983) 917–927.
27 J. F. ROBYT AND S. H. EKLUND, *Carbohydr. Res.*, 121 (1983) 279–286.
28 G. J WALKER, *Carbohydr. Res.*, 82 (1980) 404–410.
29 R. W. BAILEY, S. A. BARKER, E J. BOURNE, M. STACEY, AND O. THEANDER, *Nature*, 179 (1957) 340.
30 E. J. HEHRE, *J. Polym. Sci.*, 23 (1968) 239–244.
31 G. A. LUZIO AND R M. MAYER, *Carbohydr Res* , 111 (1983) 311–318
32 J. F ROBYT, B. K. KIMBLE, AND T F. WALSETH, *Arch. Biochem. Biophys.*, 165 (1974) 634–640.