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SYNTHESIS OF 4,6-DIDEOXYSUCROSE, AND INHIBITION STUDIES OF *Leuconostoc* AND *Streptococcus* D-GLUCANSUCRASES WITH DEOXY AND CHLORO DERIVATIVES OF SUCROSE MODIFIED AT CARBON ATOMS 3, 4, AND 6

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ABSTRACT

Starting from sucrose, 2,3,1′,3′,4′,6′-hexa-*O*-benzoyl-6-deoxy-6-iodosucrose[‡] (**1**) was synthesized. Reaction of **1** with sulfuryl chloride in pyridine gave 2,3,1′,3′,4′,6′-hexa-*O*-benzoyl-4-chloro-4,6-dideoxy-6-iodogalactosucrose (**2**). Compound **2** was treated with tributyltin hydride in toluene in the presence of a radical initiator, α,α-azobis(isobutanonitrile) (AIBN), to remove iodine and chlorine groups and give hexa-*O*-benzoyl-4,6-dideoxysucrose. Benzoyl groups were removed by sodium methoxide in methanol to give 4,6-dideoxysucrose.

Sucrose was modified at carbon atom 3, carbon atom 4, or carbon atoms 4 and 6, and these analogs were tested as inhibitors of the D-glucansucrases (D-glucosyltransferases) of *Streptococcus mutans* 6715 and *Leuconostoc mesenteroides* B-512F. Sucrose analogs used in this study are 4-deoxysucrose and 4-chloro-4-deoxygalactosucrose with *S. mutans* 6715 D-glucansucrases (GTF-S and GTF-I), and 3-deoxysucrose, 4-deoxysucrose, 4-chloro-4-deoxygalactosucrose, 6-deoxysucrose, and 4,6-dideoxysucrose with *L. mesenteroides* B-512F D-glucansucrase.

The data indicate that 3-deoxysucrose, 4-deoxysucrose, and 4-chloro-4-deoxygalactosucrose are weak noncompetitive inhibitors for B-512F dextransucrase, with K_i values of 530, 201, and 202mM respectively. For the same enzyme, 6-deoxysucrose was a strong competitive inhibitor, with K_i of 1.60mM, and 4,6-dideoxysucrose was a good competitive inhibitor, with K_i of 20.3mM. 4-Deoxysucrose was a weak noncompetitive inhibitor for both GTF-I and GTF-S, with K_i values of 672 and 608mM, respectively. 4-Chloro-4-deoxygalactosucrose was also a weak noncompetitive inhibitor for GTF-I and GTF-S with K_i values of 391 and 308mM, respectively. The inhibition data indicate that replacement of the 6-hydroxyl group

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[‡]The unprimed numerals refer to the α-D-glucopyranosyl group and the primed numerals refer to the β-D-fructofuranosyl group.

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by a hydrogen atom results in a strong competitive inhibitor, and that the hydroxyl groups at C-3 and C-4 are important in the binding of sucrose to the active sites of D-glucansucrases.

INTRODUCTION

Sucrose and α-D-glucosyl fluoride are substrates for D-glucansucrases in the synthesis of a D-glucan that has largely α-D-(1→6) D-glucose linkages[1]. Several modified α-D-glucosyl fluorides[2] and sucroses[3-6] have been synthesized in order to investigate the effects of the modification on D-glucan synthesis. Grier and Mayer[2] showed that several modified D-glucosyl fluorides are not substrates but are inhibitors. Modifications at C-6 of sucrose[‡] gave competitive inhibitors[7,18]. Binder and Robyt[7] found that 6-deoxysucrose and 6-thiosucrose are competitive inhibitors for *S. mutans* GTF-I and GTF-S. Bhattacharjee and Mayer[3] showed that 6-bromo-6-deoxysucrose and 6,6′-dichloro-6,6′-dideoxysucrose are competitive inhibitors for *Streptococcus sanguis* dextransucrase. Thaniyavarn *et al.*[4] found that methyl 6-amino-6-deoxy-α-D-glucopyranoside, methyl 6-amino-6-deoxy-α-D-mannopyranoside, methyl 6-amino-6-deoxy-β-D-glucopyranoside, 6′-amino-6′-deoxysucrose, and 6,6′-diamino-6,6′-dideoxysucrose are inhibitors for a mixture of GTF-I and GTF-S from *S. mutans* 6715. They indicated that 6,6′-diamino-6,6′-dideoxysucrose is an uncompetitive inhibitor, but gave no information about the type of inhibition of the other amino sugars they studied. Nisizawa *et al.*[8] showed that α-D-xylopyranosyl β-D-fructofuranoside is a competitive inhibitor for a mixture of *S. mutans* GTF-I and GTF-S. We have synthesized 4-chloro-4-deoxygalactosucrose[9], 4-deoxysucrose and 4,6-dideoxysucrose, and report studies on the kinetics of these sucrose analogs with *Leuconostoc mesenteroides* B-512F dextransucrase and *Streptococcus mutans* 6715 GTF-I and GTF-S.

MATERIALS AND METHODS

General methods. — Melting points were determined by using a Mel-Temp melting-point apparatus. Thin-layer chromatography (t.l.c.) was conducted on Analtech HETLC-GHLF plates. Compounds were detected by fluorescence quenching and/or sulfuric acid charring, or both. ^{13}C-Nuclear magnetic resonance (n.m.r.) spectra were obtained with a Nicolet NT-300 spectrometer at 75.5 MHz.

Leuconostoc mesenteroides B-512F dextransucrase was prepared as previously described[10]. *Streptococcus mutans* 6715 glucansucrases, GTF-I and GTF-S, were prepared as previously described[11].

Activity of the enzymes was determined by a radiochemical assay[12]. Assays of GTF-I and GTF-S were conducted in the presence of 3.3 mg of dextran T-10 per mL, at pH 6.5 and 37°; B-512F dextransucrase assays were conducted at pH 5.4 and 25°. Activity is given in International Units (IU), that is, μmol of D-glucose incorporated into D-glucan per minute. The specific activities of the enzymes were GTF-I, 1.6 IU/mg; GTF-S, 7.3; and B-512F dextransucrase, 70 IU/mg.

Sucrose analogs. — 3-Deoxysucrose[5] and 6-deoxysucrose[6] were prepared as previously reported. 4-Chloro-4-deoxygalactosucrose was prepared according to the method of Chowdhary *et al.*[9]. 4-Deoxysucrose was obtained by reduction of 4-chloro-4-deoxygalactosucrose (2 g) with tributyltin hydride (8 mL) in the presence of α,α-azobis(isobutanonitrile) (AIBN) (50 mg) in refluxing absolute ethanol (100 mL) under nitrogen gas for 46 h. Chromatography on a column of silica gel (40–140 mesh) was used to purify 4-deoxysucrose, using as eluant 11:1 (v/v) ethyl acetate–methanol. The yield of 4-deoxysucrose was 1.37 g (75.7%). 4-Deoxysucrose had previously been synthesized by the reduction of 4-chloro-4-deoxygalactosucrose using hydrogen and Raney nickel[9].

4,6-Dideoxysucrose was prepared as follows. Sulfuryl chloride (9 mL) was added dropwise to a solution of 6-deoxy-6-iodo-2,3,1′,3′,4′,6′-hexa-*O*-benzoylsucrose[7] (**1**; 10 g) in pyridine (100 mL) at −30°. The reaction was allowed to continue for 10 h at room temperature. T.l.c. in 2:1 (v/v) ether–hexane showed that all of the starting material had been converted into a faster-moving compound. The mixture (100 mL) was treated with 10% sulfuric acid (650 mL) precooled to 5°, and extracted twice with dichloromethane (500 mL). The extracts were combined and treated twice with saturated sodium hydrogencarbonate solution (250 mL), washed three times with water (250 mL), dried (magnesium sulfate), and evaporated under diminished pressure, to give solid 4-chloro-4,6-dideoxy-6-iodo-2,3,1′,3′,4′,6′-hexa-*O*-benzoylgalactosucrose (**2**; 7.12 g, 70%).

To a solution of 2.30 g of **2** in toluene (90 mL), were added tributyltin hydride[13–15] (7 mL) and (AIBN) (350 mg) and the mixture was refluxed under nitrogen for 55 h. T.l.c. with 7:1 (v/v) chloroform–light petroleum showed that all of the starting material had been converted into 4,6-dideoxy-2,3,1′,3′,4′,6′-hexa-*O*-benzoylsucrose (**3**). Toluene was removed by rotary evaporation under diminished pressure. Compound **3** was *O*-debenzoylated by addition of methanol (200 mL) and sodium methoxide (300 mg), and reaction allowed to proceed for 24 h at 25°. T.l.c. in 17:3 (v/v) acetonitrile–water then showed that all of the benzoyl groups had been removed. The solution was treated with sufficient Amberlite IRC-50 (H^+) cation-exchange resin to neutralize the sodium methoxide; the resin was removed by filtration, and the neutral filtrate was evaporated to a solid (500 mg) that was dissolved in methanol (100 mL), and adsorbed onto silica gel (50 g) by evaporation of the solvent. The solid mixture was added to the top of a dry column (3.5 × 50 cm) of Davisil 62 silica gel which was eluted with ethyl acetate (500 mL), followed by 300 mL each of 2, 4, 5, and 8% (v/v) methanol–ethyl acetate and 2 L of 10% (v/v) methanol–ethyl acetate, which eluted 4,6-dideoxysucrose (**4**). The yield was 395 mg (61% from **2**): m.p. 176–178° (dec.), $[\alpha]_D^{20}$ −61.9° (*c* 1.43, methanol).

Anal. Calc. for $C_{12}H_{22}O_9$: C, 46.45; H, 7.15. Found: C, 46.51; H, 7.03.

A proton-decoupled, ^{13}C-n.m.r. spectrum in methanol-d_4 showed that the signals of C-6 and C-4 shifted from 60.9 to 21 p.p.m. and 70.0 to 41.4 p.p.m., respectively. The proton-coupled ^{13}C-n.m.r. spectrum showed, as expected, that C-4, bearing two protons, gave a triplet ($^1J_{C\text{-}4,H\text{-}4}$ 129 Hz), and C-6, with three protons, gave a quartet ($^1J_{C\text{-}6,H\text{-}6}$ 126 Hz).

Enzyme digest conditions. — All digests of GTF-I and GTF-S were carried out at 37° in 25mM imidazolium chloride buffer (pH 6.5) containing 0.02% (w/v) of sodium azide and 2 mg/mL of Dextran T-2000 (Pharmacia). The digests of B-512F dextransucrase were carried out at 25° in 25mM sodium acetate buffer (pH 5.4) containing mM calcium chloride, sodium azide (0.1 mg/mL), and Tween 80 (0.1 mg/mL).

Kinetic digests. — All digests had a total volume of 120 μL. Sucrose concentrations employed were 5, 10, 15, and 20mM, an exception being the 3-deoxysucrose B-512F dextransucrase digest, which contained 10, 20, 30, and 40mM sucrose. The amounts of enzymes used in the digests were as follows. 3-Deoxysucrose, 12.8 mIU B-512F dextransucrase; 4-deoxysucrose, 6.56 mIU B-512F dextransucrase, 6.4 mIU GTF-I, and 5.2 mIU GTF-S; 4-chloro-4-deoxygalactosucrose, 6.4 mIU B-512F dextransucrase, 6.4 mIU GTF-I, and 5.2 mIU GTF-S; 4,6-dideoxysucrose and 6-deoxysucrose, 6.4 mIU B-512F dextransucrase. Sucrose analog concentrations are given in Figs. 1 and 2. Four time-points (6, 12, 18, and 24 min) were taken for each digest, to obtain the initial velocity. Each kinetic experiment was performed at least twice.

Assay procedure. — Digests were prepared by adding enzyme to the radioactive sucrose solution containing various amounts of sucrose analog. The specific activity of sucrose in each digest was 40 nCi/mmol. At 6, 12, 18, and 24 min, 20-μL aliquots were taken and spotted onto Whatman 3MM filter-paper squares (1.5 × 1.5 cm). These papers were immediately put into methanol and stirred for a minimum of 5 min. The papers were washed in methanol five times (to remove D-fructose, D-glucose, and unreacted sucrose). A control assay was also carried out in the absence of any sucrose analog. The filter papers were dried under a heat lamp, and the radioactivity on the papers was counted in a toluene cocktail, using a liquid scintillation spectrometer[16].

RESULTS AND DISCUSSION

Lineweaver–Burk double-reciprocal plots of the inhibition of D-glucansucrases GTF-I, GTF-S, and B-512F dextransucrase by 3-deoxysucrose, 4-deoxysucrose, 4-chloro-4-deoxygalactosucrose, 4,6-dideoxysucrose, and 6-deoxysucrose are shown in Figs. 1 and 2. The K_i values were calculated by using the slope-replot method[17] and are given in Table I. Results of previous kinetic studies of sucrose analogs with *S. mutans*[7,18], *L. mesenteroides*[18], and *S. sanguis*[3] are given in Table II.

It was found that 6-deoxysucrose is a very good competitive inhibitor, with a K_i of 1.60mM for B-512F dextransucrase. A previous study[16] had shown that 6-deoxysucrose is also a very good competitive inhibitor for GTF-I (K_i = 0.18mM) and GTF-S (K_i = 0.56mM). These results clearly show that the 6-OH group of sucrose is not crucial for the binding of sucrose to the enzymes GTF-I, GTF-S, and B-512F dextransucrase. Instead, having a hydrophobic group at C-6 increases the binding of sucrose to the enzyme, indicating that hydrogen atoms of the CH_3 group may interact with some hydrophobic groups at the active site of the enzymes.

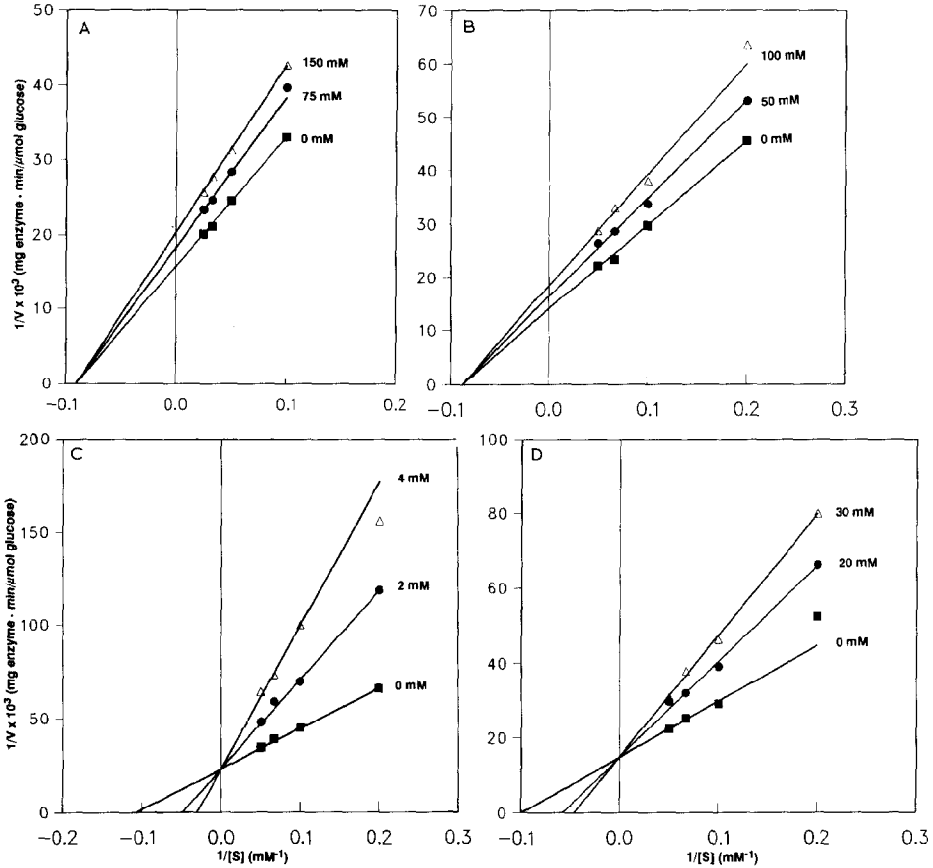

Fig. 1. Inhibition of *L. mesenteroides* B-512F dextransucrase by (A) 3-deoxysucrose, (B) 4-deoxysucrose, (C) 6-deoxysucrose, and (D) 4,6-dideoxysucrose. (Numbers beside individual curves indicate concentration of the sucrose-analog inhibitor.)

We have found that sucrose analogs modified at C-6 are generally good competitive inhibitors for D-glucansucrases. Eklund and Robyt[18] recently showed that 6-deoxy-6-fluorosucrose is a very good competitive inhibitor for GTF-I (K_i = 0.5mM), GTF-S (K_i = 1.1mM), and B-512F dextransucrase (K_i = 0.8mM). Bhattacharjee and Mayer[3] found that 6-bromo-6-deoxysucrose is a competitive inhibitor for *S. sanguis* dextransucrase (K_i = 47mM).

3-Deoxysucrose is a very weak noncompetitive inhibitor, with a K_i of 530mM for B-512F dextransucrase. This result indicates that the 3-OH group of sucrose is important for the binding of sucrose to B-512F dextransucrase. In an earlier study[7], Binder and Robyt showed that 3-deoxysucrose does not inhibit GTF-S and is a weak inhibitor for GTF-I, also indicating the importance of the 3-hydroxyl group in binding sucrose at the active sites of these enzymes.

4-Deoxysucrose is a very weak noncompetitive inhibitor for B-512F dextran-

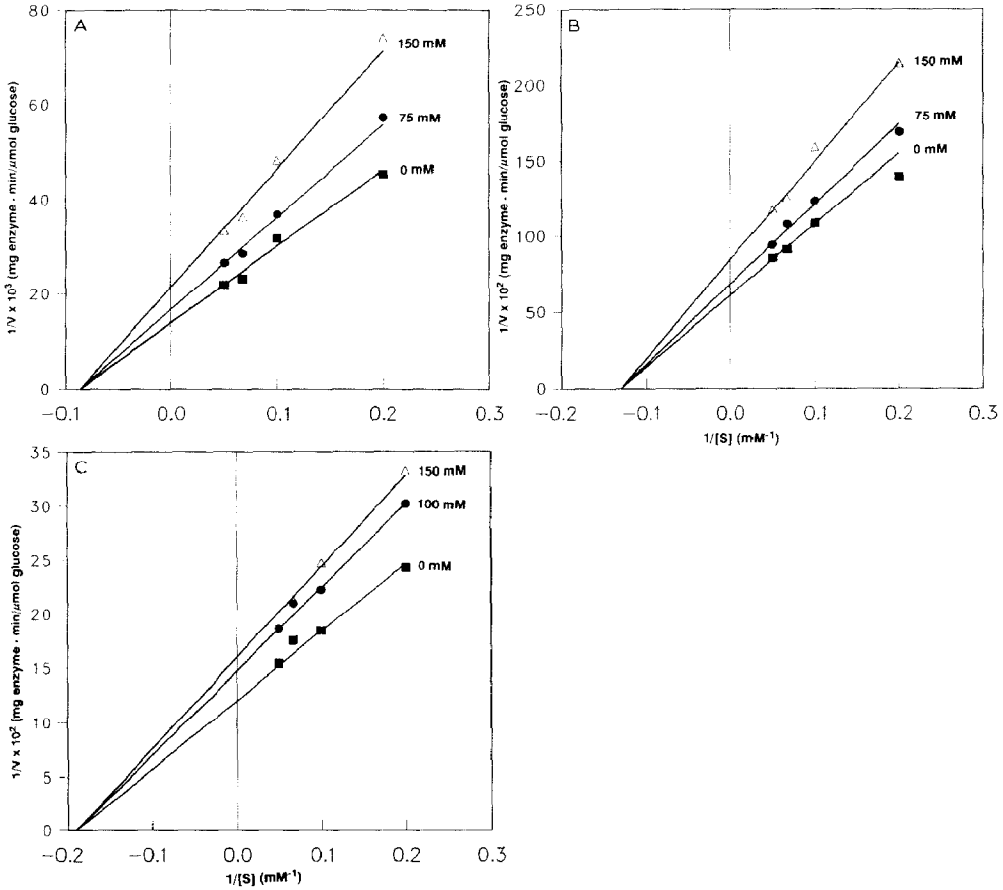

Fig. 2. Inhibition of (A) *L. mesenteroides* B-512F dextransucrase, (B) *S. mutans* 6715 GTF-I, and (C) *S. mutans* 6715 GTF-S by 4-chloro-4-deoxygalactosucrose. (Numbers beside individual curves indicate concentration of the sucrose-analog inhibitor.)

sucrase (K_i = 201mM), GTF-I (K_i = 672mM), and GTF-S (K_i = 608mM), indicating that the 4-OH group of sucrose is important for the binding of sucrose to the three enzymes. 4-Chloro-4-deoxygalactosucrose, like 4-deoxysucrose, is a very weak noncompetitive inhibitor for B-512F dextransucrase (K_i = 202mM), GTF-I (K_i = 391mM), and GTF-S (K_i = 308mM).

4,6-Dideoxysucrose was tested only with B-512F dextransucrase, and found to be a competitive inhibitor having a K_i of 20mM. This result is compatible with those of 4-deoxysucrose and 6-deoxysucrose for B-512F dextransucrase. 6-Deoxysucrose is a very good competitive inhibitor for B-512F dextransucrase, with a K_i of 1.60mM, whereas 4-deoxysucrose is a weak noncompetitive inhibitor for the same enzyme, with a K_i of 201mM. Hence, 4,6-dideoxysucrose has a K_i value that is intermediate between those of 6-deoxy- and 4-deoxy-sucrose. Absence of an OH

TABLE I

KINETIC CONSTANTS OF SUCROSE ANALOGS OBTAINED IN THIS STUDY[a,b]

Compound	*Enzyme*	K_i *(mM)*	*Type of inhibition*
3-Deoxysucrose	B-512F	530 ±21	noncompetitive
4-Deoxysucrose	B-512F	201 ±40	noncompetitive
4-Deoxysucrose	GTF-S	608 ±69	noncompetitive
4-Chloro-4-deoxygalactosucrose	B-512F	202 ±17	noncompetitive
4-Chloro-4-deoxygalactosucrose	GTF-I	391 ±26	noncompetitive
4-Chloro-4-deoxygalactosucrose	GTF-S	308 ±7	noncompetitive
6-Deoxysucrose	B-512F	1.60 ±0.02	competitive
4,6-Dideoxysucrose	B-512F	20.3 ±0.01	competitive

[a]Sucrose K_m values: B-512F dextransucrase, 10mM; GTF-I, 7.2mM; and GTF-S, 4.4mM. [b]The kinetic parameters, with standard deviations, were calculated by using the slope-replot method[17].

TABLE II

KINETIC CONSTANTS (FROM THE LITERATURE) FOR THE INHIBITION OF GLUCANSUCRASES BY SUCROSE ANALOGS

Compound	*Enzyme*	K_i *(mM)*	K_m *(mM)*	*Inhibition type*	*References*
6-Deoxy-6-fluorosucrose	*L. mesenteroides* B-512F dextransucrase	0.8	9	competitive	18
	S. mutans GTF-I	0.5	10	competitive	18
	S. mutans GTF-S	1.1	9	competitive	18
6-Deoxysucrose	*S. mutans* GTF-I	0.18	3.7	competitive	7
	S. mutans GTF-S	0.56	5.0	competitive	7
6-Thiosucrose	*S. mutans* GTF-I	3.4	3.7	competitive	7
	S. mutans GTF-S	7.3	5.0	competitive	7
3-Deoxysucrose	*S. mutans* GTF-I	36.4 305	3.7	mixed[a]	7
3-Deoxy-3-fluorosucrose	*S. mutans* GTF-S	60.7	5.0	competitive	7
	S. mutans GTF-I	40.5 153	3.7	mixed[a]	7
α-D-Allopyranosyl β-D-fructofuranoside (allosucrose)	*S. mutans* GTF-I	139	3.7	mixed[a]	7
6-Bromo-6-deoxysucrose	*S. sanguis* dextransucrase	47	5.0	competitive	3
6,6′-Dibromo-6,6′-dideoxysucrose	*S. sanguis* dextransucrase	160	5.0	mixed[a]	3
6,6′-Dichloro-6,6′-dideoxysucrose	*S. sanguis* dextransucrase	154	5.0	competitive	3
α-D-Xylopyranosyl β-D-fructofuranoside	*S. mutans* mixed GTF-I and GTF-S	2.8	4.4	competitive	8

[a]In this type of inhibition, the lines in a Lineweaver–Burk plot intersect in the second quadrant.

group at C-4 of 4,6-dideoxysucrose decreases the binding of 4,6-dideoxysucrose to B-512F dextransucrase.

In summary, it was found that 6-deoxysucrose is a very good competitive inhibitor for B-512F dextransucrase. 4,6-Dideoxysucrose is also a competitive inhibitor for B-512F dextransucrase, with a K_i value somewhat larger than that of 6-deoxysucrose due to the absence of an OH group at C-4. 4-Deoxysucrose and 4-chloro-4-deoxygalactosucrose are very weak noncompetitive inhibitors for B-512F dextransucrase, GTF-I, and GTF-S. 3-Deoxysucrose is also a very weak noncompetitive inhibitor for B-512F dextransucrase. These results show the importance of the OH group at C-3 and at C-4 for the binding of sucrose to the active sites of B-512F dextransucrase, GTF-I, and GTF-S.

At this time, the role of the 2-hydroxyl group of the D-glucosyl group of sucrose in binding to the active sites of these enzymes is not known.

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