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Neisseria perflava AMYLOSUCRASE: CHARACTERIZATION OF ITS PRODUCT POLYSACCHARIDE AND A STUDY OF ITS INHIBITION BY SUCROSE DERIVATIVES

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ABSTRACT

Neisseria perflava amylosucrase forms from sucrose a polysaccharide very similar to glycogen, except that a larger proportion of its D-glucosyl residues are in short branches. Iodine staining of samples taken during polysaccharide formation indicate that the initial product is less branched than that formed at longer times. This glycogen-like polysaccharide has an estimated molecular mass range of 1 MD to 20 MD. Sucrose derivatives modified at C-3 (3-deoxysucrose and α-D-allopyranosyl β-D-fructofuranoside), C-6 (6-deoxysucrose and 6-deoxy-6-fluorosucrose), and both C-4 and C-6 (4,6-dideoxysucrose) were tested as inhibitors of amylosucrase. Derivatives modified at C-6 were potent competitive inhibitors, with K_i values of 6.2 ±0.3mM (6-deoxysucrose) and 0.50 ±0.06mM (6-deoxy-6-fluorosucrose). The K_M value of sucrose is 26.5 ±4.6mM. Sucrose derivatives modified at C-3 were not significantly inhibitory over the concentration range tested. 4,6-Dideoxysucrose gave an unusual, non-competitive inhibition, in that, increasing its concentration did not produce a commensurate increase in the level of inhibition, which instead appeared to approach a limit. None of these sucrose derivatives was a substrate for amylosucrase, nor were they glycosyl donors to maltotriose.

INTRODUCTION

The synthesis of a glycogen-like polysaccharide by *Neisseria perflava* amylosucrase (EC 2.4.1.4, sucrose:1,4-α-D-glucan 4-α-glucosyltransferase) is unique among enzymic syntheses of amylopolysaccharides, as the D-glucan is formed directly from the substrate sucrose, without the intervention of α-D-glucosyl nucleoside diphosphate intermediates. The similarity of this reaction to that catalyzed by *Leuconostoc mesenteroides* and *Streptococcus mutans* dextransucrases

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(EC 2.4.1.5, sucrose:1,6-α-D-glucan 6-α-glucosyltransferase) made comparison of the kinetics and inhibitory behavior of amylosucrase of interest.

Amylosucrase was discovered by Hehre and Hamilton[1] in 1946. Subsequent studies by Hehre and coworkers[2–7] and others[8,9] examined the behavior of this constitutive enzyme from *N. perflava,* showing that it produces a glycogen-like polysaccharide directly from α-D-glucopyranosyl fluoride[6], as well as from sucrose. Their studies also showed[7] that amylosucrase is strongly inhibited by sucrose concentrations >200mM, and is activated by the presence of its native polysaccharide or a variety of other amylopolysaccharides[7]. Amylosucrase has been postulated to have a role in the formation of dental caries[10,11]. However, since these investigations were conducted, this enzyme has received very little additional attention.

The present work further characterizes the polysaccharide produced by amylosucrase, and investigates the inhibitory behavior of various sucrose derivatives.

EXPERIMENTAL

Carbohydrates. — [U-^{14}C]Sucrose was obtained from Schwarz–Mann, Inc. (Spring Valley, NY). Allosucrose (α-D-allopyranosyl β-D-fructofuranoside), 3-deoxysucrose, 4,6-dideoxysucrose, 6-deoxysucrose, and 6-deoxy-6-fluorosucrose were all previously synthesized in this laboratory[12–15]. Non-labeled and ^{14}C-labeled polysaccharides from amylosucrase were made, by using 100mM sucrose in 100mM sodium maleate buffer (pH 7.0) at 35°, followed by ethanol precipitation. The recovered polysaccharide was dissolved in the same buffer to make a 200-mg/mL solution that was purified by dialysis against the same buffer. Thin-layer chromatography (t.l.c.) showed no residual sucrose or D-fructose present. No amylosucrase activity was detected in the purified polysaccharide preparation. T-series dextrans were obtained from Pharmacia (Uppsala, Sweden).

Enzymes. — *N. perflava* 19-34 was obtained from Professor E. J. Hehre, and was grown in a 5-L fermentor, following the procedure of Okada and Hehre[7]. After centrifugation, the cells were suspended in ~3 mL of 100mM sodium maleate buffer, pH 7.0, and rapidly disrupted by three passages through a French pressure cell precooled to 4°. The remaining steps were all performed at 4°. The suspension was centrifuged for 40 min at 120,000*g*, and the supernatant liquor was recovered by decantation. This solution was treated overnight with an equal volume of an 80% saturated ammonium sulfate solution at pH 6.4. Following centrifugation, the sediment was dissolved in 3 mL of 50mM sodium maleate buffer (pH 7.0) containing 25mM 2-mercaptoethanol and 0.02% sodium azide. This solution was desalted by passage through a column of Sephadex G-10, using the same buffer as eluant. The enzyme fractions, which emerged at the void volume, were collected and pooled. The protein concentration of this solution was measured as 11 mg/mL, using the method of Lowry *et al.*[16], standardized with bovine serum albumin. The enzyme was kept at −20°, and retained full activity for over six months; it constituted the

enzyme used in the study. Further purification was attempted by size-exclusion chromatography [Bio-Gel P-150 (Bio-Rad Laboratories, Richmond, CA)], ion-exchange chromatography (DEAE-cellulose), and absorption onto cross-linked starch–glycogen[17], but did not yield stable, active enzyme. Therefore, further purification was not performed.

The specific activity of the enzyme used was 0.50 U/mg of protein, where one unit of activity is defined as 1 μmol.min^{-1} of D-glucose incorporated into D-glucan at pH 7.0 and 35° in the presence of ~200 μg/mL of non-radiolabeled native amylosucrase polysaccharide, using an initial concentration of 100mM [U-^{14}C]-sucrose. Details of the radiochemical assay are presented.

This enzyme preparation showed no amylolytic activity based on reaction with 1% starch solution, as measured by reducing value using the ferricyanide method[18].

Liquid scintillation counting. — ^{14}C-Labeled carbohydrate samples adsorbed onto paper or plastic-backed silica were placed face up in 10 mL of toluene scintillation fluid in 20 mL scintillation vials, and the amount of radiolabel was measured[19].

Thin-layer chromatography. — Analysis for the presence of malto-oligosaccharides was performed by t.l.c. Samples were spotted onto plates of Whatman K5 silica gel (Whatman Chemical Separation, Inc., Clifton, NJ), and dried. Separation was effected by a single ascent of 37:40:23 (v/v/v) ethyl acetate–methanol–water, and the compounds were detected by spraying with 4:1 (v/v) methanol–sulfuric acid followed by heating for 10 min at 120°. For determination of higher oligosaccharides (d.p. 8–15), three ascents of 2:3:5 (v/v/v) nitromethane–water–1-propanol were used.

Determination of the molecular weight of the polysaccharide. — A sample of amylosucrase-produced polysaccharide in 100mM sodium maleate buffer, pH 7.0, was passed over a column (10 mm × 500 mm) of Bio-Gel A-150m that had been pre-equilibrated with the same buffer. Samples (1 mL) were collected, and analyzed by the phenol–sulfuric acid method for total carbohydrate[20]. Dextrans of mol. wt. 465,000, 2×10^6, and 150×10^6 were used as standards.

Permethylation analysis of the polysaccharide. — Dr. M. E. Slodki of the Northern Regional Research Center of the U. S. Department of Agriculture (Peoria, IL) kindly performed this analysis[21].

Isoamylase digestion of the polysaccharide. — ^{14}C-Labeled *N. perflava* polysaccharide (~100 μg) made from [U-^{14}C]sucrose and separated from residual sucrose and D-fructose by dialysis, was incubated with 100 units of *Pseudomonas amyloderamosa* isoamylase [EC 3.2.1.68, glycogen 6-glucanohydrolase][22] (Sigma Chemical Co., St. Louis, MO) in 50mM sodium acetate buffer, pH 4.0, for 6 h at 35°. Following digestion, the sample was placed on a piece of Whatman 3MM paper (200 × 500 mm), and eluted with a descending flow of 7:3 (v/v) 1-propanol–water for 24 h. Following drying, an autoradiogram of the paper was made. The radioactive compounds were excised, and counted by liquid scintillation spectrometry. Six

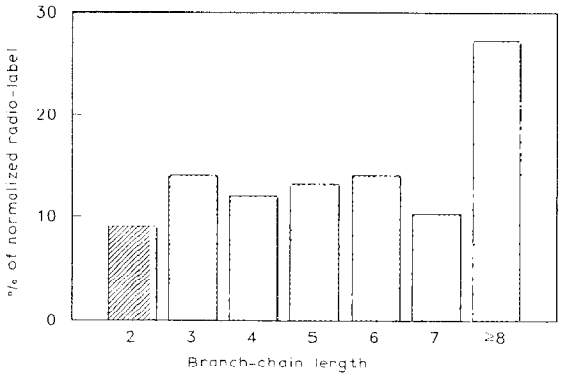

Fig. 1. Mol % of radiolabel in isoamylase-digested polysaccharide branches. (The fraction having d.p. ≥8 was normalized by dividing the amount of radioactivity in the fraction by 10; the other fractions were normalized by dividing the amount of radioactivity in each fraction by its respective chain-length.)

products were resolved from the origin. The two fastest running were eluted from the paper with water, dried, and dissolved in 10 μL of water. When analyzed by t.l.c., and compared to malto-oligosaccharide standards, these solutions were found to correspond to maltose and maltotriose. From this result, the four other products were assumed to be maltotetraose through maltoheptaose. The distribution of radiolabel, normalized by d.p., are presented in Fig. 1. The d.p. of the largest fraction (≥8) was taken as 10 for this Figure.

Polysaccharide iodine staining. — A reaction mixture, consisting of 1.5 mL of 100mM sucrose in 100mM sodium maleate buffer, pH 7.0, with 100 μL of amylosucrase, was incubated at 35°; samples (100 μL) were withdrawn at various times and added to 1 mL of distilled water containing 100 μL of 0.2% I_2 + 2% KI aqueous solution. The spectral absorbance was measured at various wavelengths from 400 to 600 nm.

Reaction conditions. — All reactions were conducted at 35° in 50–100mM sodium maleate, pH 7.0, containing 0.02% sodium azide. Except as noted, reaction mixtures also contained ~200 mg/mL of non-labeled, amylosucrase-produced polysaccharide to eliminate D-glucan synthesis lag[7].

Measurement of enzyme activity. — Various concentrations of [U-^{14}C]sucrose were incubated with 20 μL of enzyme (0.1 U) solution in 300-μL reaction digests. Periodically, 25-μL aliquots were removed, and deposited on 1-cm^2 pieces of Whatman 3MM filter paper. The papers were washed five times in methanol, to remove methanol-soluble label, followed by heterogeneous, liquid scintillation counting to quantitate the formation of methanol-insoluble, labeled product[23].

Inhibitor kinetics reactions. — A series of reactions using different concentrations of [U-^{14}C]sucrose and sucrose derivatives was performed, in order to test the ability of the latter, individually, to inhibit D-glucan formation by amylosucrase. The concentration ranges used were 2.5–50mM [U-^{14}C]sucrose, 10–50mM

allosucrose, 0.1–50mM 3-deoxysucrose, 1–30mM 4,6-dideoxysucrose, 0.1–50mM 6-deoxysucrose, and 0.5–5mM 6-deoxy-6-fluorosucrose. 4.6-Dideoxysucrose was also tested as an activator in the absence of added native polysaccharide.

Identification of substrates, acceptors, and glycosyl donors. — Each of the sucrose derivatives was tested to determine if it could act as a substrate in polysaccharide synthesis. For this, 100-μL reaction mixtures consisting of 50mM solutions of each derivative combined with 20 μL of amylosucrase (0.1 U) were prepared without added native polysaccharide or other carbohydrate acceptors, and were incubated for 6 d at 35° in the presence of 0.02% azide. Polymer formation was tested by visual observation of the formation of opalescence, and by t.l.c.

Each sucrose derivative was also tested as a glycosyl donor to maltotriose. A series of 300-μL mixtures, 50mM in sucrose derivative and 50mM in maltotriose, and containing 20 μL of amylosucrase (0.1 U), was incubated. Samples were withdrawn at 1, 2, 4, and 24 h, and analyzed by t.l.c. for the presence of higher d.p. malto-oligosaccharides.

Furthermore, dextran was tested as an acceptor of D-glucosyl groups from [U-^{14}C]sucrose. A mixture of 500 μL of a 1% solution of dextran T-10 (Pharmacia) and 50mM [U-^{14}C]sucrose was incubated with 20 μL of amylosucrase (0.1 U) for 1 h. The product mixture as loaded onto a column (10 mm × 500 mm) of Bio-Gel A-0.5m, and eluted with 100mM sodium maleate buffer, pH 7.0. Samples (1 mL) were collected, and analyzed for total carbohydrate by the phenol–sulfuric acid method[20]. In addition, 50-μL aliquots of each sample were spotted onto a 1-cm^2 piece of 3MM Whatman paper, and counted by liquid scintillation spectrometry. Comparison of the elution profiles for the two methods were made, in order to determine if significant amounts of label were incorporated into the dextran fraction.

Transglycosylation. — Reaction mixtures of 50mM of each individual malto-oligosaccharide, maltose through maltoheptaose, were incubated with amylosucrase for 48 h to detect transglycosylation products. Isomaltose and isomaltotriose were also tested. Reaction samples were analyzed by t.l.c., and compared to malto-oligosaccharide standards.

Transglycosylation activity was quantitated by using 50mM ^{14}C-labeled maltotetraose under the same reaction conditions. Samples taken at various times were separated by t.l.c., using a plastic-backed t.l.c. plate (Silica Gel 80, MCB Reagents, Gibbstown, NJ). An autoradiogram of the plate was made, and the maltotetraose spots were excised and counted by liquid scintillation spectrometry.

RESULTS

Characterization of amylosucrase polysaccharide. — The estimated molecular weight range of the amylopolysaccharide measured by size-exclusion chromatography was ~1 to 20 × 10^6. Methylation analysis of this polysaccharide indicate that ~90% of the linkages were (1→4), with the remaining 10% being (1→6) branch

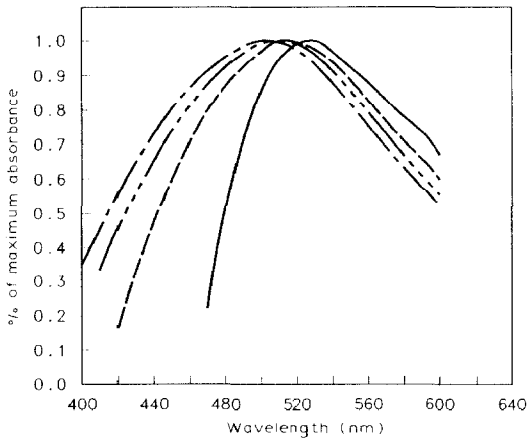

Fig. 2. Change of absorbance of iodine stain spectra of amylosucrase glycogen with reaction time. [Key: ——, 15 min; · —— ·, 45 min; – – –, 90 min; and —— –, 360 min.]

linkages. Addition of iodine–iodide solution produced a reddish-brown complex similar to that of iodine-stained glycogen. Absorption measurements of the iodine-stained complex yielded a general spectral shift to lower wavelengths with increasing reaction time, including a shift of λ_{max} from 530 to 500 nm (see Fig. 2). Quantitation of the radiolabeled products from isoamylase debranching of radiolabeled polysaccharide by paper chromatography and liquid scintillation counting indicated that ~70 mol% of the D-glucosyl residues were contained in branch chains of seven or fewer residues (see Fig. 1). Oligosaccharide branches up to d.p. 12 were detectable by t.l.c.; however, they could not be resolved by paper chromatography.

Amylosucrase kinetic parameters with sucrose substrate. — As previously noted[7], amylosucrase is inhibited by concentrations of sucrose >100mM. Initial-rate data fit the standard substrate inhibition model, assuming a second non-productive sucrose-binding site (see Fig. 3).

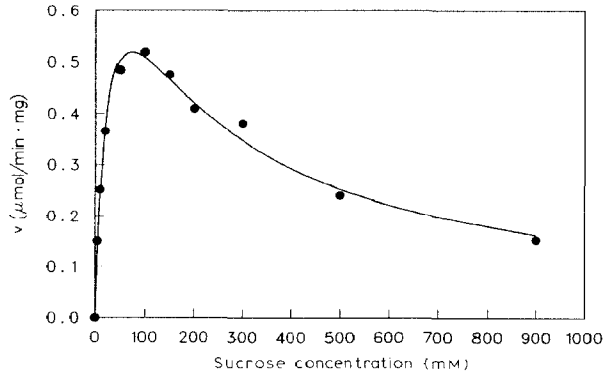

Fig. 3. Dependence of amylosucrase activity on sucrose concentration.

$$v = \frac{V_m[S]}{K_M + [S] + [S]^2/K_s}$$

Values calculated by non-linear regression, using the SAS NONLIN program (SAS Institute Inc., Cary, NC), for V_m, K_M, and K_s, were 0.90 ±0.08 μmol.min^{-1} per mg of protein, 26.5 ±4.6mM, and 201 ±35mM, respectively, where the second value presented is the standard deviation. These values are similar to those obtained by Okada and Hehre[7] (K_m = 17mM, K_s = 305mM). Dextran T-10 was not an acceptor of D-glucosyl groups from sucrose, as no significant amount of label was found in the dextran elution fraction. The observation by Okada and Hehre[7] that the enzyme is activated by its native polysaccharide was also confirmed.

Inhibition by sucrose derivatives. — Various sucrose derivatives were tested

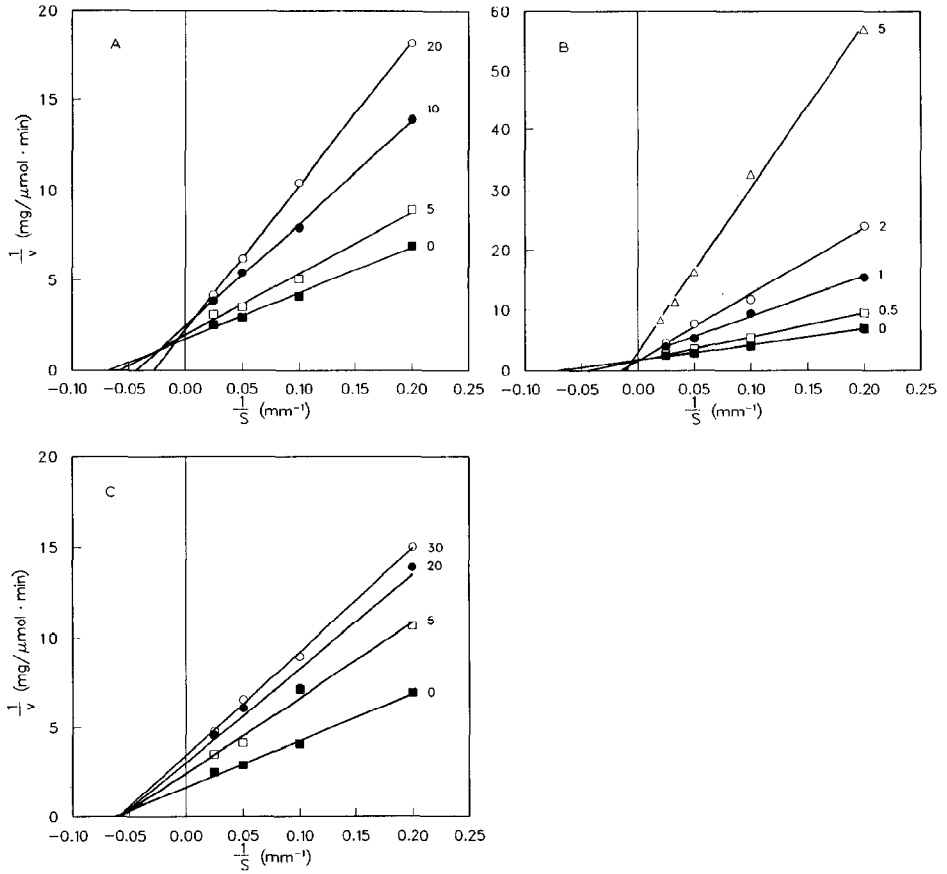

Fig. 4. Lineweaver–Burk plots of D-glucose incorporation into D-glucan by the reaction of amylosucrase with [U-^{14}C]sucrose in the presence of the following inhibitors: (a) 6-deoxysucrose, (b) 6-deoxy-6-fluorosucrose, and (c) 4,6-dideoxysucrose. [Concentrations on the plots are those of the inhibitors in mM.]

TABLE I

KINETIC CONSTANTS OF INHIBITORY EFFECTS OF SUCROSE DERIVATIVES ON AMYLOSUCRASE

Compound	*Kinetic constant (mM)*	*Type of inhibition*
Sucrose (K_m)	26.5 ±4.6	
Sucrose (K_s)	201 ±35	substrate
6-Deoxy-6-fluorosucrose (K_i)	0.50 ±0.06	competitive
6-Deoxysucrose (K_i)	6.2 ±0.3	competitive
4,6-Dideoxysucrose (K_i)	22.5 ±4.0	non-competitive
3-Deoxysucrose		[a]
α-D-Allopyranosyl β-D-fructofuranoside		[a]

[a]Did not give significant inhibition over the concentration ranges tested.

as inhibitors of D-glucan formation from sucrose. Fig. 4 shows Lineweaver–Burk plots of D-glucose incorporation into D-glucan by reaction of amylosucrase with [U-^{14}C]sucrose in the presence of these derivatives. K_i values were calculated by non-linear regression, using the SAS NONLIN program. Table I summarizes the kinetic and inhibition parameters obtained from these analyses. Neither 3-deoxysucrose nor allosucrose gave significant inhibition over the concentration ranges tested. Both 6-deoxysucrose and 6-deoxy-6-fluorosucrose proved to be strong competitive inhibitors, with K_i values of 6.2 ±0.3mM and 0.50 ±0.06mM, respectively. 4,6-Dideoxysucrose was also an inhibitor, but it showed unusual behavior. The appearance of the reciprocal plot (see Fig. 4) implies that 4,6-dideoxysucrose is a noncompetitive inhibitor. However, increasing the inhibitor concentration did not produce a corresponding rise in the level of the inhibition, which appeared to approach a maximum value (see Fig. 5). Fitting the data for this

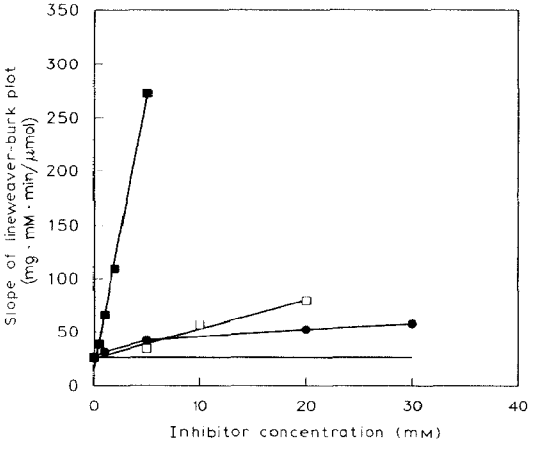

Fig. 5. Effect of inhibitor concentration on the slopes of the Lineweaver–Burk plots. [Key: ■, 6-fluorosucrose; □, 6-deoxysucrose; ●, 4,6-dideoxysucrose; and ——, no inhibitor.]

reaction using a non-competitive model, $v = K_i V_m [S]/(K_M + [S])(K_i + [I])$ yielded a K_i value of 22.5 ±4.0mM.

Reactivity of sucrose derivatives and malto-oligosaccharides. — None of the sucrose derivatives was a substrate for amylosucrase, as determined visually by the absence of opalescence in the reaction mixture and by t.l.c. Similarly, none was a glycosyl donor to maltotriose. However, a small amount of disproportionation of maltotriose to D-glucose and maltopentaose was observed. Further investigation of this disproportionation reaction was carried out by incubating a series of malto-oligosaccharides (from maltose to maltoheptaose) with amylosucrase. Isomaltose and isomaltotriose were also investigated. T.l.c. analysis of the digests of maltotriose and maltotetraose indicated that maltosyl or maltotriosyl units, respectively, were exclusively transferred, to form maltopentaose or maltoheptaose with the release of D-glucose. Faint spots corresponding to maltoheptaose appeared at long maltotriose-digestion times. Incubation of maltopentaose, maltohexaose, and maltoheptaose with amylosucrase led to the initial formation of oligomers that were one D-glucosyl unit longer and shorter than the original oligosaccharide. These were followed by further disproportionation products of both lower and higher chain-length. Maltose, isomaltose, and isomaltotriose did not undergo disproportionation reactions.

Amylosucrase and transglycosylase activities, using enzyme previously heated for 10 min to 50°, were measured at 35° and pH 7.0, to determine if these activities were related. The transglycosylase activity was ~1000-fold less than that of the amylosucrase at 35° and pH 7.0. The results were compared to the activity of the unheated enzyme. The heated enzyme retained 25% of the amylosucrase activity while losing all transglycosylase activity. The unheated enzyme had no measurable activity at pH 4.0 and 35°.

DISCUSSION

Amylosucrase and *S. mutans* dextransucrase have many points of similarity. Both are bacterial α-D-glucosyltransferases that catalyze the formation of specific, high-molecular-weight D-glucans from sucrose. Both are inhibited by high concentrations of sucrose and are activated by the presence of native polysaccharide. The present work provides information showing several additional similarities, as well as some mechanistic differences between the two enzymes.

The present study confirmed the structural similarity to glycogen of the amylopolysaccharide produced by amylosucrase. Its degree of branching (10%) and iodine-staining color (reddish-brown) are closest to those of various glycogens[24–26]. It differs in having branch chains somewhat shorter than those of most glycogens and a λ_{max} of 500 nm, which is slightly higher than that of most glycogens, but considerably lower than that of amylopectin[24]. The iodine-stain absorbance spectra of the amylosucrase glycogen shows a shift to shorter wavelengths with reaction time. This is due to increased branching, which implies that

branching is not the intially predominant reaction during polymerization[27], but is similar to the branching reaction of dextransucrase and occurs by an acceptor mechanism by segments of the synthesized amylodextrin chains[28,29].

In the case of 3-modified compounds, no inhibitory or synthetic activity catalyzed by amylosucrase was observed. This implies that the 3-position is critical to the binding of substrate to the enzyme, similar to *S. mutans* glucansucrases[30]. However, in the case of dextransucrase, 3-deoxysucrose is both a weak inhibitor and a glycosyl donor.

6-Deoxysucrose and 6-deoxy-6-fluorosucrose both possess strong amylosucrase inhibitory behavior, similar to that of dextransucrase[14,31], implying that binding occurs and involves the 6-hydroxyl group. Because the main linkage in dextran is α-D(1→6), it is not surprising that the 6-modified sucroses bind well to the active site of dextransucrase but do not react. However, the difference in the K_i values of 6-deoxysucrose and 6-deoxy-6-fluorosucrose for amylosucrase was not as great as with dextransucrase. Replacement of the hydroxyl group by a hydrogen atom gives a sucrose derivative that is a competitive inhibitor, with a K_i of ~6mM. Substitution of a fluorine atom for the hydroxyl group, which more closely mimics the electronic nature of the oxygen atom in the hydroxyl group, increases the effectiveness of inhibition approximately tenfold, yielding K_i = 0.5mM and indicating that the hydroxyl group on C-6 of sucrose acts as a hydrogen-bond acceptor.

6-Deoxysucrose and 6-deoxy-6-fluorosucrose were not substrates or glycosyl donors to maltotriose for either enzyme. The inability of these derivatives to act as substrates for the enzyme suggests that the presence of the hydroxyl group at C-6 of sucrose affects catalysis, as well as being important to binding. A possible explanation is that the modifications alter the binding position of the sucrose derivative in the active site. This binding change leads to enhanced binding due to steric and electronic forces, but so positions the glycosidic bond that it cannot undergo reactions catalyzed by the enzyme.

Amylosucrase D-glucan product is mainly composed of D-(1→4)-glucosidic linkages, and thus the 4-position is quite important for both binding and catalysis. Comparing the inhibitory behavior of 6-deoxysucrose and 4,6-dideoxysucrose indicates that the substitution of hydrogen for the hydroxyl group on C-4 lessens the inhibitory activity at high concentrations of inhibitor, and changes the type of inhibition from competitive to noncompetitive. However, increasing the inhibitor concentration does not produce a commensurate rise in inhibitory activity. The extent of inhibition appears to level off with increasing inhibitor concentration (see Fig. 5), implying that a different mechanism of inhibition may be occurring. A possible cause of this kind of behavior is that an alternative binding mode exists when the concentration becomes higher, which affects the catalytic process. Interestingly, 4,6-dideoxysucrose appears to have similar, unusual inhibitory action on dextransucrase[15].

Because of the similarity in behavior of 4,6-dideoxysucrose with both amylosucrase and dextransucrase, and because of the modification at the 4- and 6-posi-

tions, where the glycosidic linkage is respectively formed by amylosucrase and dextransucrase, it is tempting to speculate that the unusual inhibitory effects are related to binding at the active site. An alternative possible explanation is that the sucrose derivative preferentially binds at the sucrose inhibitory binding site to lessen the inhibitory effect. The addition of 4,6-dideoxysucrose in reaction mixtures of sucrose and amylosucrase with and without added polysaccharide does not eliminate the lag in activity observed in the absence of the exogenous polysaccharide. Therefore, the lowered inhibitory effect at higher inhibitor concentrations is probably not due to binding of the modified sucrose at the polysaccharide site.

Hitz *et al.*[32] found similar binding affinity in a sucrose-transport protein from soybean, using modified phenyl D-glucopyranoside derivatives and sucrose modified in the D-fructosyl moiety.

The presence of transglycosylation activity is apparently not directly related to amylosucrase activity, in that heating the enzyme mixture before reaction affects the two activities differently. Therefore, we consider that the transglycosylase activity observed is not an intrinsic function of the amylosucrase, but, rather, is caused by a separate transglycosylase present in the enzyme mixture. Although the enzyme used was not purified to homogeneity, due to its apparent lability upon purification[7,17], the preparation was devoid of alpha amylase, glucosidase, and debranching activity. The only significant catalytic activity observed was glycogen synthesis from sucrose.

In summary, the general behavior of *N. perflava* amylosucrase and both *L. mesenteroides* and *S. mutans* dextransucrases towards sucrose and the sucrose derivatives used in these studies is quite similar, given the different glycosidic linkages in the product polysaccharides. It is interesting to speculate on the similarity in mechanism, particularly with respect to branching, as the bacterial species that produce the two enzymes occupy the same environment and use the same substrate. In both types of enzymes, the 3-position is critical for binding. However, dextransucrase is capable of transferring glycosyl groups from sucrose derivatives modified at this position, whereas amylosucrase does not exhibit this ability. Inhibitions by sucrose derivatives modified at C-6 indicate that this position is important for binding and catalysis in both enzymes. For amylosucrase, the absence of a hydroxyl group, or substitution of a fluorine atom, strongly enhances binding, indicating that both steric and hydrogen-bonding forces may play an important role at this position. Simultaneous substitution of hydrogen for the hydroxyl groups on C-4 and C-6 of sucrose produced an unusual inhibitory effect for both enzymes. A possible interpretation may be that different modes of binding exist, effecting the observed inhibitory behavior.

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