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p-NITROPHENYL α-D-GLUCOPYRANOSIDE, A NEW SUBSTRATE FOR GLUCANSUCRASES*

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ABSTRACT

p-Nitrophenyl α-D-glucopyranoside has been shown to be a substrate for the glucansucrases of various strains of *Leuconostoc mesenteroides* and *Streptococcus mutans*. The products from a digest of *p*-nitrophenyl α-D-glucopyranoside with *L. mesenteroides* B-512F dextransucrase were found to include dextran, a series of *p*-nitrophenyl isomaltodextrin glycosides, and *p*-nitrophenyl nigeroside. The kinetics of the reaction were non-Michaelis–Menten, possibly because *p*-nitrophenyl α-D-glucopyranoside has a dual role in the reaction as both a D-glucosyl donor and acceptor.

INTRODUCTION

The glucansucrases produced by several species and strains of *Leuconostoc* and *Streptococcus* synthesize D-glucans, utilizing sucrose as a high-energy, D-glucosyl donor. Earlier work has shown that glucansucrases will also polymerize the D-glucosyl groups from α-D-glucopyranosyl α-L-sorbofuranoside[1], *O*-β-D-galactopyranosyl-(1→4)-β-D-fructofuranosyl α-D-glucopyranoside (lactulosucrose)[2], and α-D-glucopyranosyl fluoride[3]. All three have an α-D-glucosidic linkage of energy similar to, or higher than, that of sucrose.

While looking for a possible inhibitor of the dextransucrase produced by *Leuconostoc mesenteroides* B-512F, it was found that *p*-nitrophenyl α-D-glucopyranoside (PNPG) was hydrolyzed by this enzyme. In order to determine whether this hydrolysis was caused by a contaminating activity or by the dextransucrase, the products of the reaction were isolated and identified. PNPG incubated with B-512F dextransucrase gave acceptor products similar to those produced when the enzyme was incubated with sucrose and an appropriate acceptor[4,5] and a high-molecular-weight D-glucose polymer which was shown to be identical to B-512F dextran by both ^{13}C-n.m.r. spectroscopy and dextranase hydrolysis.

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EXPERIMENTAL

Enzymes. — *Leuconostoc mesenteroides* B-512F dextransucrase was obtained from culture supernatant fluid, which was concentrated, treated with dextranase to remove exogenous dextran, and chromatographed on DEAE-cellulose[6].

L. mesenteroides B-742 mixed glucansucrases were obtained directly from culture supernatant fluid, which was dialyzed and concentrated[7].

L. mesenteroides B-1355 alternansucrase was prepared, and separated from other glucansucrases, by the method of Côté and Robyt[8].

Streptococcus mutans 6715 was grown on the medium described by Ciardi *et al.*[9]. After removal of cells, and concentration by using a Millipore Pellicon filtration system, the glucansucrases were eluted from a Bio-Gel A-15m column[10]. The two glucansucrases were separated by ion-exchange chromatography on DEAE-cellulose[11] in 20mM sodium phosphate buffer, pH 6.8, containing 0.02% of sodium azide by using a gradient of 0 to 0.2M sodium chloride. Further purification of the glucansucrases was accomplished by a second ion-exchange chromatography step[12] using DEAE-Bio-Gel A with the same phosphate buffer and a 0 to 0.2M sodium chloride gradient. The solution of the glucosyltransferase, which produces soluble polysaccharide (GFT-S), was then dialyzed, and concentrated by filtration through an Amicon XM-100 membrane. The enzyme responsible for synthesis of insoluble polysaccharide (GTF-I) was taken through a Sephadex G-50, affinity-chromatography step[12].

Glucansucrase activity, determined by a radiochemical assay using [U-^{14}C]sucrose[13], is given in International Units (IU), *i.e.*, μmol of D-glucose incorporated per min into D-glucan at pH 5.2 and 25°, except for GTF-S and GTF-I, which were assayed at 37° in buffer containing 3.3 mg of T-10 dextran/mL.

Exo-isomaltodextranase was prepared from *Arthrobacter globiformis* T-6 by the method of Sawai *et al.*[14].

Dextranase, an endodextranase produced by a strain of *Penicillium funiculosum*, was obtained from Sigma Chemical Co., St. Louis, MO.

Carbohydrates. — [U-^{14}C]Sucrose was obtained from New England Nuclear, Boston, MA, and *p*-nitrophenyl α-D-glucopyranoside (PNPG), from Sigma Chemical Co. Isomalto-oligosaccharides were prepared by partial hydrolysis of commercial B-512F dextran (Sigma Chem. Co.) with acid: a solution of dextran (15 g) in 0.3M trifluoroacetic acid (500 mL) was heated on a steam bath for 2 h. Nigerose was prepared by acetolysis of *L. mesenteroides* B-1355 glucan-S (alternan)[15], and purified by chromatography on silica gel.

p-Nitro-[6-^{3}H]phenyl α-D-glucopyranoside was prepared by a method similar to that used for cyclodextrins by Weselake and Hill[16]. Other sugars were obtained commercially.

Chromatography. — Thin-layer chromatography (t.l.c.) was performed on Whatman K5F 0.25-mm silica gel plates (Whatman Chemical Separations, Inc., Clifton, NJ). T.l.c. plates were developed in one of the following solvent systems:

(*A*) 4:1:3:2 (v/v/v/v) acetonitrile–nitroethane–ethanol–water; or (*B*) 17:3 (v/v) acetonitrile–water. Detection was achieved both by quenching of u.v. fluorescence and by charring by spraying with 20% sulfuric acid in methanol followed by heating for 10 min at 110°.

The Bio-Gel P-30 column (2.5 × 75 cm) was eluted with water, and 4.2-mL fractions were collected; the Bio-Gel A-1.5m column (1 × 30 cm) was eluted with water, and 0.90-mL fractions were collected.

Liquid chromatography (l.c.) at 24 MPa was performed in a Waters Associates model ALC/GPC-201 liquid chromatograph, using a Whatman Partisil M9/50 polar aminocyano column. Solvent systems were either 9:1 or 4:1 (v/v) acetonitrile–water with a flow rate of 4 mL/min. Detection was achieved both by refractive index and by u.v. absorbance at 300 nm.

Enzyme-digest conditions. — All digestions were conducted in 20mM pyridine–acetic acid buffer, pH 5.2, 2 mM calcium chloride, 0.01% sodium azide, and 0.1% Tween 80, at 25°, unless stated otherwise.

Preparation and characterization of PNPG products. — B-512F dextransucrase (0.1 mL, 2 IU) was added to 33.0mM PNPG in buffer (1 mL). T.l.c. plates were spotted with 25 μL of the digest at 4-h intervals for 44 h, and then at 8–12 h intervals for a total of 146 h. The t.l.c. plates were developed with one ascent in solvent *A*.

A second digest containing 20mM PNPG in buffer (5 mL) was treated with B-512F dextransucrase (20 IU/mL, 0.5 mL) for 7 d at 30°, concentrated to 2 mL, and applied to a Bio-Gel P-30 column. Each fraction was analyzed for total carbohydrate by the phenol–sulfuric acid method[17], as well as for presence of the *p*-nitrophenyl group by absorbance measurements at 300 nm. The void-volume fractions were applied to the Bio-Gel A-1.5m column, and each fraction was reanalyzed for total carbohydrate.

A third digest contained 31mM PNPG in buffer (25 mL) to which was added B-512F dextransucrase (125 IU/mL, 40 μL); it was allowed to react for 5 d at 25°. The dextran formed was precipitated with ethanol (2 vol.), and the suspension centrifuged. The supernatant fluid was concentrated to 1 mL, placed in a Bioanalytical Systems filtering centrifuge-tube, and centrifuged through a 0.45-μm (pore size) aqueous membrane. The low-molecular-weight products in the filtrate were separated by 24-MPa l.c. The precipitated dextran, dissolved in water (2 mL), was eluted in the void volume of a Bio-Gel P-30 column; it was again precipitated with ethanol (2 vol.) and dried, to yield 40 mg of dextran.

The products isolated by l.c. were evaporated to dryness, and each was redissolved in buffer (0.5 mL); aliquots (100 μL) were treated with exo-isomaltodextranase[14,18–20] at 30°, and the hydrolysis products were examined by t.l.c. with one ascent of solvent *A*. Partial hydrolysis of the acceptor product not susceptible to the exo-isomaltodextranase was conducted by treating 0.2 mL with 0.4M trifluoroacetic acid (0.8 mL) for 40 min at 90° in a sealed vial that had been flushed with nitrogen;

the reaction mixture was lyophilized, and the residue dissolved in water (0.1 mL). The products were analyzed by t.l.c., using three ascents of solvent *B*.

The dextran (40 mg) was dissolved in deuterium oxide (2 mL) and the ^{13}C-n.m.r. spectrum was recorded with a JEOL FX 90Q ^{13}C-n.m.r. spectrometer in the Fourier-transform, proton-decoupled mode, at 22.5 MHz and 80°. An aliquot of the dextran in buffer was also treated with dextranase, and the hydrolysis was monitored by t.l.c., using two ascents in solvent *A*.

Reaction kinetics. — For a series of concentrations ranging from 12 to 31mM of [6-^{3}H]PNPG in buffer, 1 mL was added to 0.1 mL of B-512F dextransucrase (20 IU/mL). Aliquots were removed at various times for determining *p*-nitrophenol and formation of dextran. *p*-Nitrophenol was determined by adding 50 μL of the digest to 2.5 mL of 0.5M sodium carbonate and measuring the absorbance[21] at 415 nm; dextran formation was determined by spotting 40 μL of the enzyme digest onto a square (1.5 × 1.5 cm) of Whatman 3MM paper which was washed 5 times with methanol, followed by liquid scintillation counting[13]. The velocity of a 28mM [U-^{14}C]sucrose digest was also determined by measuring [^{14}C]dextran formation, using one-tenth as much B-512F dextransucrase.

Because [^{3}H]PNPG was quenched more in heterogeneous counting than [^{3}H]dextran, the extra quenching factor was determined by counting standard aliquots of [^{3}H]PNPG and [^{3}H]dextran on paper squares in toluene cocktail, and in solution in 1,4-dioxane cocktail.

The relative velocities of various glucansucrases were determined by adding 100-μL aliquots of 33.1mM PNPG in buffer to 100 μL of each of the following enzymes: *L. mesenteroides* B-1355 alternansucrase (0.23 IU/mL), *L. mesenteroides* B-742 mixed glucansucrases (0.6 IU/mL), *Streptococcus mutans* 6715 GTF-S (40 IU/mL), or *S. mutans* 6715 GTF-I (10.4 IU/mL). GTF-S and GTF-I were incubated at 37°. At various times, 50-μL aliquots were analyzed for release of *p*-nitrophenol from PNPG.

RESULTS

The t.l.c. of the time course of the reaction of *p*-nitrophenyl α-D-glucopyranoside (PNPG) with B-512F dextransucrase is shown in Fig. 1. Polysaccharide formation is observed early in the reaction along with traces of acceptor products. As time progresses, a series of isomalto-oligosaccharides (d.p. 2 to 9) is produced. The enzyme digest was chromatographed on Bio-Gel P-30 (see Fig. 2 for the elution profile). Three u.v.-absorbing peaks were observed, two of which contained carbohydrate. The first (small) u.v. peak, at the void volume, may be due either to light-scattering caused by the dextran, or to the presence of a *p*-nitrophenyl group at the (originally reducing) end of the polysaccharide. The second u.v. peak, which corresponded to the total included volume, contained all of the low-molecular-weight PNPG acceptor products, as well as some free D-glucose. The third u.v. peak corresponded to *p*-nitrophenol, which had some affinity for the column packing.

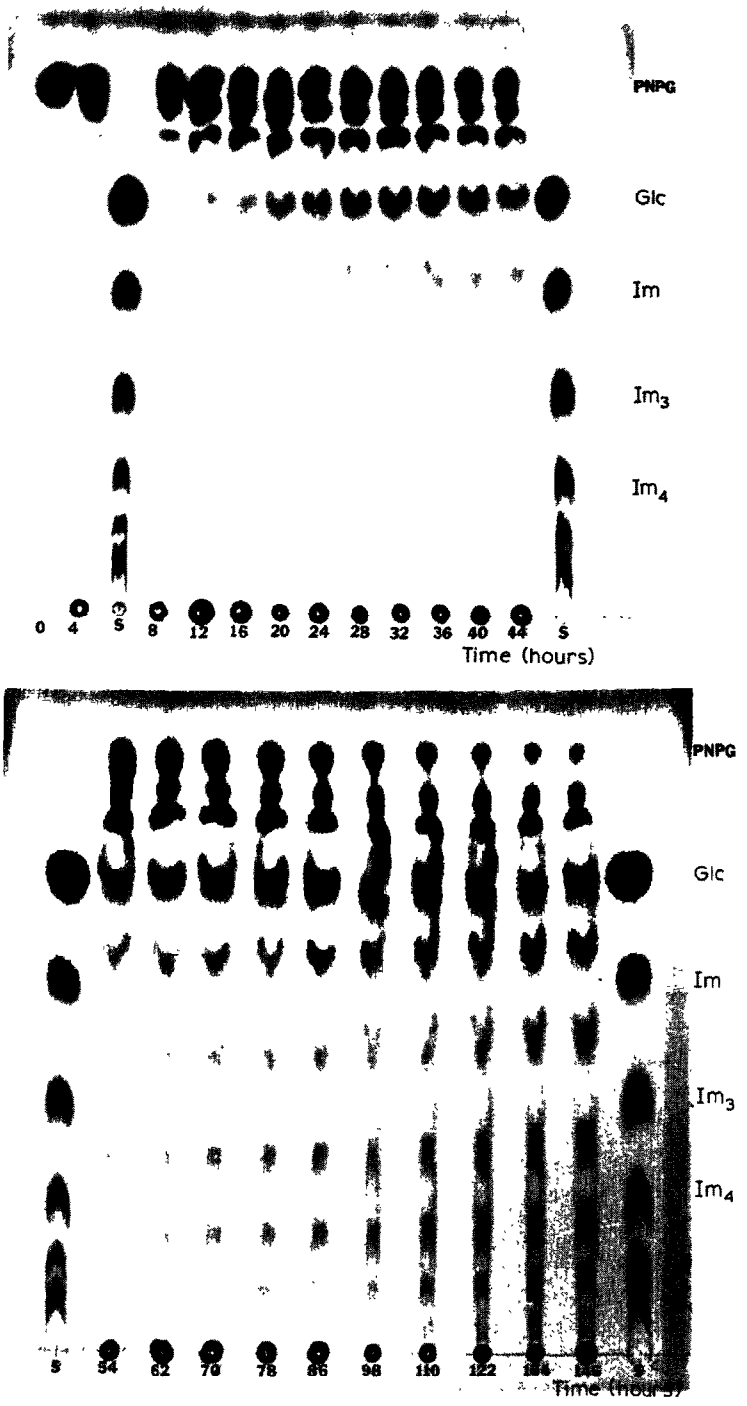

Fig. 1. T.l.c. of the time course of the reaction of PNPG with B-512F dextransucrase. [The digest (30 μL) was spotted at each of the time points shown. S is an isomaltodextrin series. T.l.c. was conducted for one ascent in solvent *A* at room temperature.]

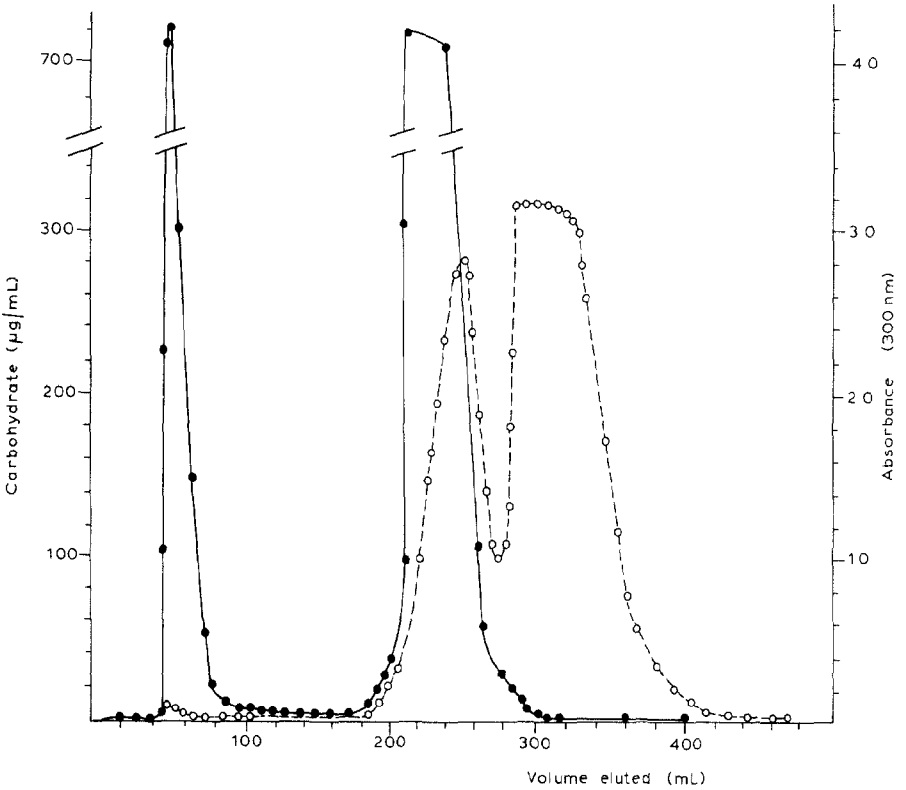

Fig. 2. Bio-Gel P-30 elution-profile of the products from a B-512F dextransucrase digest of PNPG. [The column was 2.5 × 75 cm, eluted with water; 4.2-mL fractions were collected. ——— = total carbohydrate; - - - - - - = u.v. absorbance at 300 nm.]

The void-volume peak from the Bio-Gel P-30 column was chromatographed on a Bio-Gel A-1.5m column (see Fig. 3). Its elution profile is similar to that of native B-512F dextran, although a little more tailing from the void volume peak was observed with the dextran produced from PNPG.

The individual, low-molecular-weight products were isolated by 24-MPa l.c. with 4:1 (v/v) acetonitrile–water (see Fig. 4). All but one (peak 3) of the series of peaks was u.v.-absorbing, and it had the same mobility as free D-glucose. Peak 1 had multiple components, and so it was rechromatographed in 9:1 (v/v) acetonitrile–water, to give the profile in Fig. 5.

Table I shows the results of exo-isomaltodextranase treatment of the acceptor products. Prolonged treatment with the isomaltodextranase released *p*-nitrophenol if the number of residues in α-(1→6) linkage was even, and *p*-nitrophenyl α-D-glucopyranoside if the number of residues was odd. Only peak 3 in Fig. 4, and peaks 1C and 1D in Fig. 5, were unaffected by isomaltodextranase. Peak 1C had the same chromatographic mobility as the starting material (PNPG), with a slight shoulder that probably corresponds to the β anomer present in the commercial

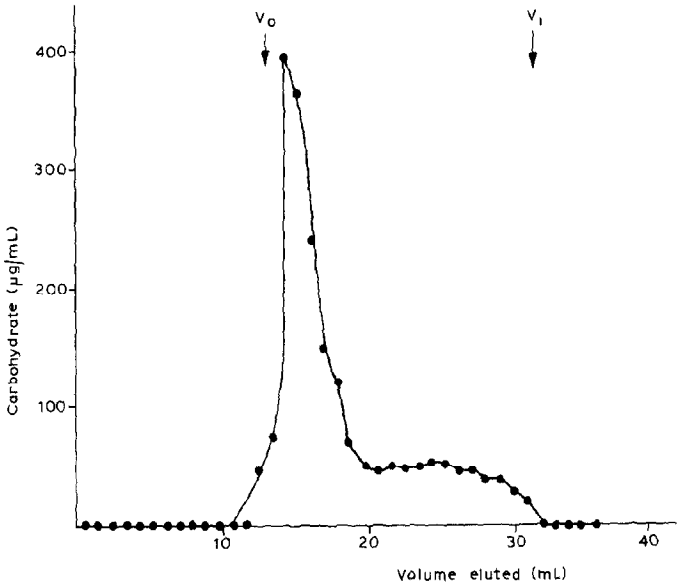

Fig. 3. Bio-Gel A-1.5m elution-profile of the void-volume fraction from the Bio-Gel P-30 column (see Fig. 2). [The column was 1 × 30 cm, eluted with water; 0.90-mL fractions were collected.]

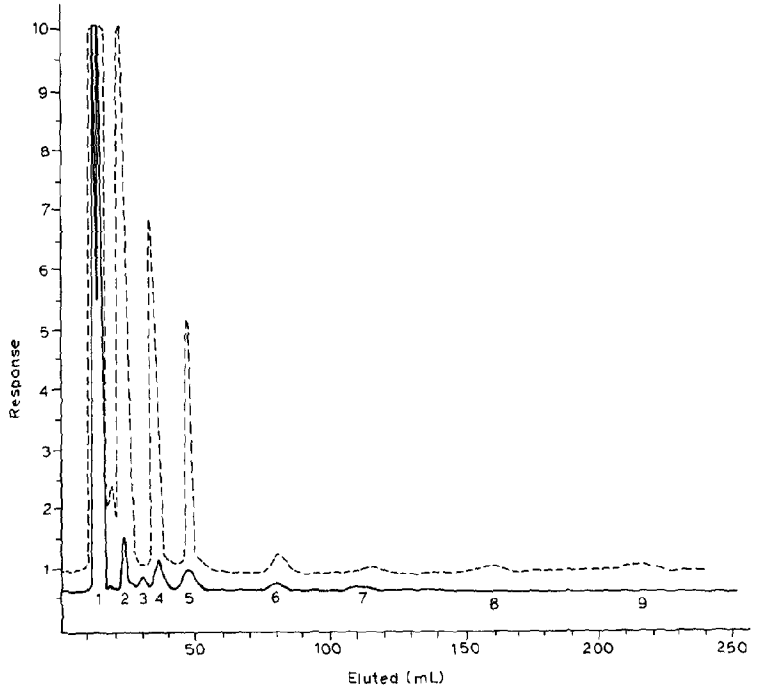

Fig. 4. 24-MPa l.c. profile of PNPG acceptor-products formed by B-512F dextransucrase action on PNPG. [The column was a Partisil M9/50 PAC, eluted with 4:1 (v/v) acetonitrile–water at a flow rate of 4 mL/min. ——— = refractive index; - - - - - - = u.v. absorbance at 300 nm.]

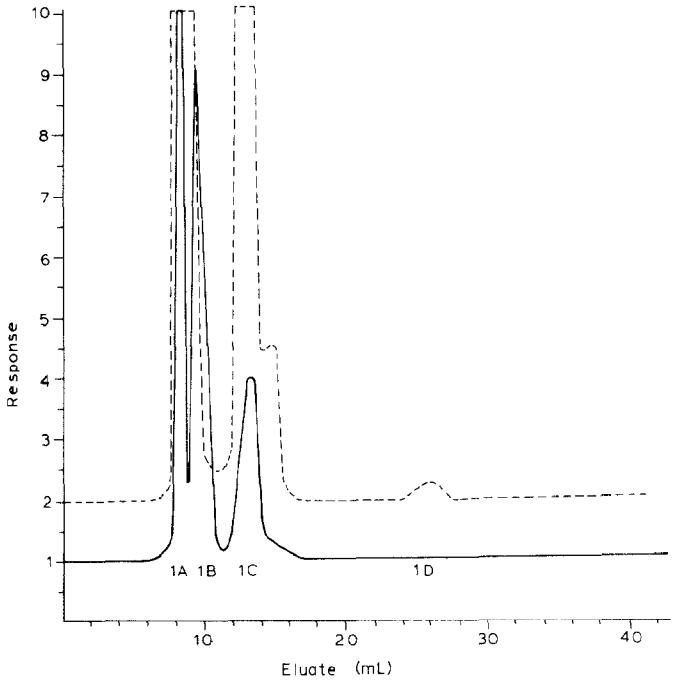

Fig. 5. 24-MPa l.c. of peak 1 from Fig. 4, using the same column and flow rate. [Solvent system was 9:1 (v/v) acetonitrile–water. ——— = refractive index; - - - - - - = u.v. absorbance at 300 nm]

TABLE I

THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF THE *Arthobacter globiformis* T-6 ISOMALTODEXTRANASE DIGESTION-PRODUCTS OF *p*-NITROPHENYL α-D-GLUCOPYRANOSIDE ACCEPTORS

Peak	*Digestion products*[a]				*Structure*
	PNP	*PNPG*	*IM*	*Glc*	
1A	+	–	–	–	PNP
1B	–	–	–	–	solvent front
1C	–	+	–	–	PNPG
1D	–	–	–	–	PNP nigeroside*
2	+	–	+	–	PNP isomaltoside
3	–	–	–	+	Glc
4	–	+	+	–	PNP isomaltotrioside
5	+	–	+	–	PNP isomaltotetraoside
6	–	+	+	–	PNP isomaltopentaoside
7	+	–	+	–	PNP isomaltohexaoside
8	–	+	+	–	PNP isomaltoheptaoside
9	+	–	+	–	PNP isomaltooctaoside

[a]Key: + = detected by t.l.c., – = not detected by t.l.c., PNP = *p*-nitrophenol, or *p*-nitrophenyl, PNPG = *p*-nitrophenyl α-D-glucopyranoside, IM = isomaltose, Glc = D-glucose, and * = identified by partial hydrolysis with acid.

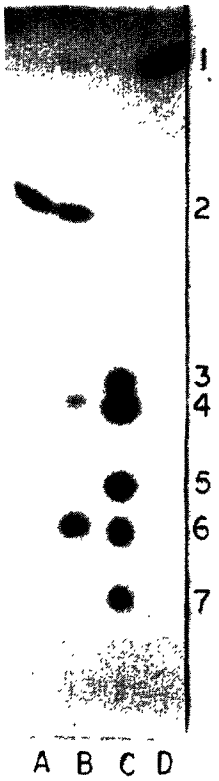

Fig. 6. Partial acid-hydrolysis products of peak 1D (see Fig. 5). [Lane B had 10 μL of partially acid-hydrolyzed peak 1D spotted. Lanes A, C, and D had standards spotted as follows: 1, PNPG; 2, peak 1D; 3, D-fructose; 4, D-glucose; 5, sucrose; 6, nigerose; 7, isomaltose.]

preparation. Peak 1D was identified by partial hydrolysis with acid (see Fig. 6). Partial hydrolysis of 1D with acid gave a light spot having the same mobility as PNPG (the starting material), a spot corresponding to D-glucose, and a spot having the same mobility as nigerose, which established the structure of the component in peak 1D as *p*-nitrophenyl nigeroside. Thus, the acceptor products constituted a homologous series ranging from *p*-nitrophenyl isomaltoside through *p*-nitrophenyl isomalto-octaoside, with the additional, α-(1→3)-linked *p*-nitrophenyl nigeroside. Higher-d.p. acceptor-products, although observed in t.l.c., were not present in large enough quantities to be isolable by l.c.

The dextran produced from PNPG gave the ^{13}C-n.m.r. spectrum in Fig. 7. Only six resonances are observed, and they correspond to those reported for dextran produced by B-512F dextransucrase[22]. Dextranase hydrolysis of the PNPG-produced dextran gave mainly isomaltose and isomaltotriose, with small amounts of higher-d.p., branched products, apparently identical to the dextranase products from native B-512F dextran.

The kinetics of the reaction of PNPG are given in Figs. 8 and 9. The rate of

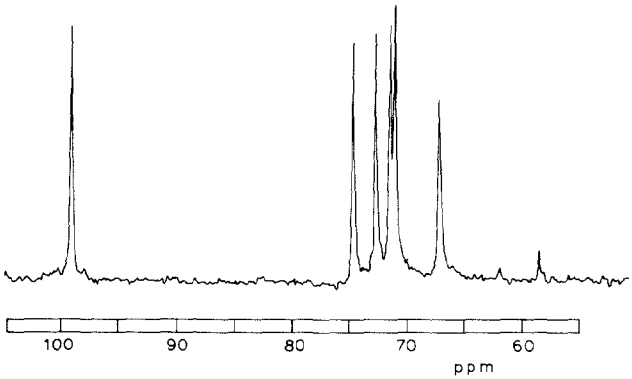

Fig. 7. ^{13}C-N.m.r. spectrum of the dextran produced from PNPG by B-512F dextransucrase. [Sample concentration, 20 mg/mL; temperature 80°.]

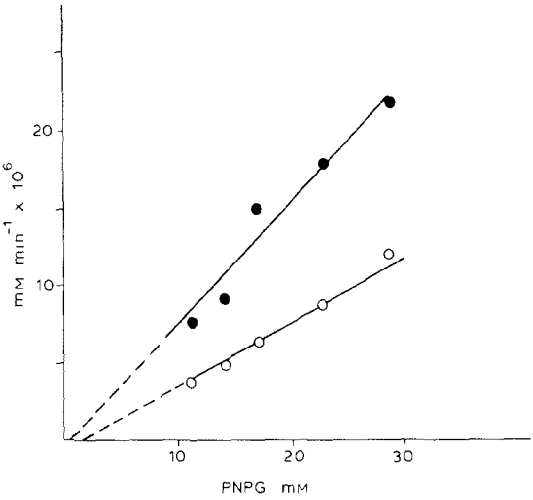

Fig. 8. Plot of velocity *vs.* substrate for B-512F dextransucrase incubated with various concentrations of [^{3}H]PNPG at 25°. (—○—○—○— = D-[^{3}H]glucose incorporation into methanol-insoluble polysaccharide; —●—●—●— = *p*-nitrophenol released.)

release of *p*-nitrophenol is 1.9 times that of incorporation of D-glucose into methanol-insoluble dextran; this is not surprising, as a considerable amount of PNPG acceptor products, which are methanol-soluble, are also formed. We were unable to obtain either the K_m or the V_{max} values from the Michaelis plot (Fig. 9), because of negative y-axis intercepts which most probably are due to PNPG acting as both an acceptor and a donor, giving mixed-order kinetics. It might be expected that, using higher concentrations of PNPG, the plots would show curvature; but, because PNPG has a relatively low solubility in water (33mM at 25°), it was necessary to use 30mM PNPG as the highest concentration.

PNPG was also shown to be a less effective substrate than sucrose. A comparison of the initial rates of incorporation of D-glucose into dextran from PNPG

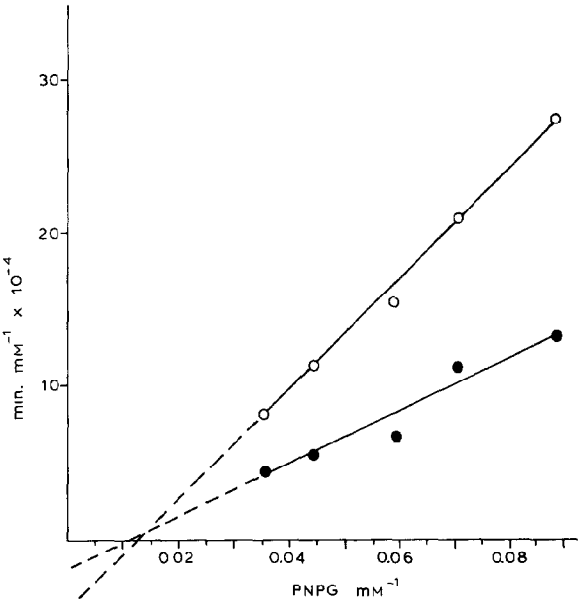

Fig. 9. Michaelis plot of B-512F dextransucrase action on [^{3}H]PNPG at 25°. (—○—○—○— = D-[^{3}H]glucose incorporation into methanol-insoluble polysaccharide; —●—●—●— = *p*-nitrophenol released.)

and sucrose, each at 28mM, showed that the rate of D-glucose incorporation into dextran from sucrose was 158 times that from PNPG.

Table II gives a relative comparison of the reaction of various glucansucrases with PNPG. PNPG was utilized most rapidly by *L. mesenteroides* B-512F dextransucrase, and reacted ~0.03 times as fast with *S. mutans* 6715 GTF-S. *L. mesenteroides* B-742 glucansucrases (a mixture of two types of glucansucrase), *L. mesenteroides* B-1355 alternansucrase, and *S. mutans* 6715 GTF-I were intermediate between the two other enzymes.

TABLE II

RELATIVE RATES OF RELEASE OF *p*-NITROPHENOL BY GLUCANSUCRASES

Enzyme source	*Enzyme*	*Relative rate of release of* p*-nitrophenol* ($\mu mol.min^{-1}.IU^{-1}$)
L. mesenteroides B-512F	Dextransucrase	7.45×10^{-3}
L. mesenteroides B-742	Glucansucrases (mixture)	5.56×10^{-3}
L. mesenteroides B-1355	Alternansucrase	1.68×10^{-3}
S. mutans 6715	Glucansucrase (GTF-I)	1.58×10^{-3}
S. mutans 6715	Glucansucrase (GTF-S)	2.40×10^{-4}

DISCUSSION

p-Nitrophenyl α-D-glucopyranoside has been shown to be a substrate for glucansucrases. The rate of formation of dextran by *Leuconostoc mesenteroides* B-512F dextransucrase from PNPG is ~0.6% of that from sucrose at 28mM. Sucrose will react to form some minor, acceptor products, leucrose and isomaltulose[23], as well as some free D-glucose[24,25], when no other acceptors are present, but, at most, these account for only 10% of the D-glucose while the other 90% is incorporated into dextran[26]. On the other hand, when PNPG reacts with B-512F dextransucrase, it gives only 53% incorporation of D-glucose into methanol-insoluble dextran; the rest is released as free D-glucose, or is incorporated into acceptor products. Therefore, the actual rate of cleavage of PNPG, measured by release of *p*-nitrophenol, is 1% of that of sucrose.

Even though PNPG is a less effective substrate, the fact that the *p*-nitrophenol, which is released by the reaction, can be monitored spectrophotometrically[21,27] makes it a useful alternative substrate for assaying glucansucrase activity. Unfortunately, the kinetics of the PNPG reaction with glucansucrases are complex, because of the two different reaction-pathways that occur simultaneously. Whereas sucrose, the natural substrate, reacts rapidly, mainly to form polysaccharide, PNPG reacts at a much lower rate, to form not only polysaccharide but also a series of low-molecular-weight, acceptor products. The latter may be the cause of the non-Michaelis–Menten kinetics observed for the reaction.

The formation of *p*-nitrophenyl nigeroside in the acceptor reactions is of interest, because, normally, when D-glucose is added as an acceptor, dextransucrase forms only a linear, homologous series of isomalto-oligosaccharides having no α-(1→3) linkages[28], although α-(1→3) branch linkages are formed when dextran is added as an acceptor[29]. Why PNPG forms an α-(1→3)-linked acceptor product with itself may have some bearing on how the *p*-nitrophenyl group affects the binding of D-glucosyl groups to the glucansucrase binding-sites.

One final, interesting observation is that the various D-glucosyltransferases are able to utilize PNPG as a substrate to different extents. What this may mean as far as differences in reaction mechanism or substrate binding are concerned has yet to be determined. The fact that PNPG is a relatively poor substrate for *S. mutans* 6715 GTF-S and GTF-I may explain why PNPG had earlier been reported[30] not to be a substrate for a crude preparation of *S. mutans* 6715 glucansucrases.

In summary, *p*-nitrophenyl α-D-glucopyranoside reacts with *L. mesenteroides* B-512F dextransucrase as both a D-glucosyl donor and acceptor, to give high-molecular-weight dextran and low-molecular-weight oligosaccharide acceptor-products. The course of the reaction may be readily monitored by absorbance measurements[21,27] at 415 nm. The kinetics are complex, and non-Michaelis–Menten, but linear, initial velocities may be obtained. Although PNPG is not as effective a substrate as sucrose, and reacts to different extents with different glucansucrases, it may be used as a convenient, alternative substrate for glucansucrase as-

says, as the method of detection is simple and straightforward, and this substrate is commercially available. Thus, PNPG has several advantages over the other alternate substrate, α-D-glucopyranosyl fluoride[3], which must be synthesized by using hydrogen fluoride[31], is not particularly stable in aqueous solutions, and requires a fluoride-electrode for detection[32].

REFERENCES

1 J. C. MAZZA, A. AKGERMAN, AND J. R. EDWARDS, *Carbohydr. Res.*, 40 (1975) 402–406.
2 E. J. HEHRE AND H. SUZUKI, *Arch. Biochem. Biophys.*, 113 (1966) 675–683.
3 D. S. GENGHOF AND E. J. HEHRE, *Proc. Soc. Exp. Biol. Med.*, 140 (1972) 1298–1301.
4 H. J. KOEPSELL, H. M. TSUCHIYA, N. N. HELLMAN, A. KAZENKO, C. A. HOFFMAN, E. S. SHARPE, AND R. W. JACKSON, *J. Biol. Chem.*, 200 (1952) 793–801.
5 F. YAMAUCHI AND Y. OHWADA, *Agric. Biol. Chem.*, 33 (1969) 1295–1300.
6 A. W. MILLER AND J. F. ROBYT, unpublished results.
7 G. L. CÔTÉ AND J. F. ROBYT, *Carbohydr. Res.*, 119 (1983) 141–156.
8 G. L. CÔTÉ AND J. F. ROBYT, *Carbohydr. Res.*, 101 (1982) 57–74.
9 J. E. CIARDI, A. J. BEAMAN, AND C. L. WITTENBERGER, *Infect. Immun.*, 18 (1977) 237–246.
10 A. SHIMAMURA, H. TSUMORI, AND H. MUKASA, *Biochim. Biophys. Acta*, 702 (1982) 72–80.
11 G. R. GERMAINE, S. K. HARLANDER, W.-L. S. LEUNG, AND C. F. SCHACHTELE, *Infect. Immun.*, 16 (1977) 637–648.
12 R. M. HAMELIK AND M. M. MCCABE, *Biochem. Biophys. Res. Commun.*, 106 (1982) 875–880.
13 G. R. GERMAINE, C. F. SCHACHTELE, AND A. M. CHLUDZINSKI, *J. Dent. Res.*, 53 (1974) 1355–1360.
14 T. SAWAI, K. TORIYAMO, AND K. YANO, *J. Biochem. (Tokyo)*, 75 (1974) 105–112.
15 K. MATSUDA, H. WATANABE, K. FUJIMOTO, AND K. ASO, *Nature*, 191 (1961) 278.
16 R. J. WESELAKE AND R. D. HILL, *Carbohydr. Res.*, 104 (1982) 334–337.
17 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
18 M. TORII, K. SAKAKIBARA, A. MISAKI, AND T. SAWAI, *Biochem. Biophys. Res. Commun.*, 70 (1976) 459–464.
19 T. SAWAI, T. TOHYAMA, AND T. NATSUME, *Carbohydr. Res.*, 66 (1978) 195–205.
20 T. SAWAI, S. OHARA, Y. ICHIMI, S. OKAJI, K. HISADA, AND N. FUKAYA, *Carbohydr. Res.*, 89 (1981) 289–299.
21 F. MALEY, *Carbohydr. Res.*, 64 (1978) 279–282.
22 A. J. BENISI AND J. T. GARIG, *Carbohydr. Res.*, 53 (1977) 278–283.
23 F. H. STODOLA, E. S. SHARPE, AND H. J. KOEPSELL, *J. Am. Chem. Soc.*, 78 (1956) 2514–2518.
24 A. GOODMAN, R. M. WEIL, AND K. G. STERN, *J. Biol. Chem.*, 217 (1955) 977–985.
25 G. A. LUZIO AND R. M. MAYER, *Carbohydr. Res.*, 111 (1983) 311–318.
26 J. F. ROBYT AND S. H. EKLUND, *Carbohydr. Res.*, 121 (1983) 279–286.
27 K. AIZAWA, *J. Biochem. (Tokyo)*, 30 (1939) 89–100.
28 K. L. SMILEY, M. E. SLODKI, J. A. BOUNDY, AND R. D. PLATTNER, *Carbohydr. Res.*, 108 (1982) 279–283.
29 J. F. ROBYT AND H. TANIGUCHI, *Arch. Biochem. Biophys.*, 174 (1976) 129–135.
30 J. J. THOMAS, B. J. THOMAS, C. WILKINSON, E. HYNES, M. A. FINN, AND R. H. JONES, *FEMS Microbiol. Lett.*, 4 (1978) 27–30.
31 F. MICHEEL AND A. KLEMER, *Adv. Carbohydr. Chem.*, 16 (1961) 85–103.
32 W. R. FIGURES AND J. R. EDWARDS, *Carbohydr. Res.*, 48 (1976) 245–253.