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Control of the synthesis of dextran and acceptor-products by *Leuconostoc mesenteroides* B-512FM dextransucrase †

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ABSTRACT

In the maltose-acceptor reaction of *Leuconostoc mesenteroides* B-512FM dextransucrase, some of the D-glucose moieties of sucrose are diverted from the synthesis of dextran and are transferred to the nonreducing end of maltose to form panose. Glucose is also transferred to panose and to subsequent acceptor products to give a homologous series of isomaltosyl dextrins attached α-(1 → 6) to maltose. Three experimental parameters were studied to obtain quantitative information about the yield and distribution of acceptor products and the yield of dextran: (*a*) the ratio of maltose to sucrose, (*b*) the concentration of maltose and sucrose, and (*c*) the amount of enzyme. The reactions were run with [^{14}C]sucrose and the amount of each acceptor product and the amount of dextran synthesized were determined for (*a*), (*b*), and (*c*) by TLC separation and measurement of the radioactivity with a PhosphorImager. It was found that an increase in the ratio of maltose to sucrose increased the *amount* of acceptor products with a concomitant decrease in the synthesis of dextran. Further, as the ratio was increased, the *number* of acceptor-products decreased. When the concentrations of maltose and sucrose were increased and the ratio was maintained at 1:1, there also was a decrease in the amount of dextran and an increase in the amount of acceptor-products. In addition, there was a decrease in the amount of dextran and an increase in the amount and number of acceptor-products when the amount of enzyme was increased. The first acceptor-product can be exclusively obtained without the formation of any dextran, by using a specific ratio and concentration of maltose and sucrose and a specified amount of enzyme.

INTRODUCTION

Dextransucrase catalyzes the synthesis of dextran without the need of a primer[1] by initially forming two covalent glucosyl–enzyme complexes in which OH-6 of one of the glucose units attacks C-1 of the other to form an α-(1 → 6) glycosidic bond, giving an isomaltose unit attached to the enzyme[2]. This is the beginning of a growing dextran chain that is covalently attached to the active site of the enzyme and elongated by an insertion mechanism[3]. In the presence of an acceptor, such as

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maltose, the maltose binds to an acceptor binding-site and blocks the transfer of glucose into dextran[4]. The OH-6 group of the nonreducing end of the maltose attacks the covalently linked glucose or dextran, giving transfer of these units to maltose[5]. When glucose is transferred, the acceptor-product, panose results. Panose itself is an acceptor to give a tetrasaccharide, 6^2-*O*-α-isomaltosylmaltose; this tetrasaccharide is also an acceptor to give a pentasaccharide, 6^2-*O*-isomaltotriosylmaltose, etc. There, thus, is produced a homologous series of acceptor-products having isomaltodextrins attached to O-6 of the nonreducing end of maltose[2,5]. These acceptor products are designated here as P3, P4, P5, etc., indicating products with dp 3, 4, 5, etc. Robyt and Eklund[6] showed that maltose was the best acceptor in the dextransucrase reaction and that the amount of D-glucose diverted from dextran into acceptor products was dependent on the ratio of maltose to sucrose. They found that the amount of dextran dropped rapidly as the ratio of maltose to sucrose was increased. At a concentration of 80 mM sucrose and a ratio of maltose to sucrose of 1:1, four acceptor-products of dp 3 to 6 were formed with the formation of 18% dextran. When the ratio was increased to 2.5:1, the proportion of dextran dropped to 9%.

To obtain a better quantitative understanding of the acceptor reaction, we have studied the effects of varying the ratio of maltose to sucrose over a wide range from 1:5 to 100:1, using a constant amount of total carbohydrate (100 mM), a constant amount of sucrose (50 and 100 mM) with varying amounts of maltose, a constant ratio of maltose to sucrose (1:1) at varying concentrations from 1.25 to 300 mM, and different amounts of enzyme acting with a maltose to sucrose ratio of 1:1 at different concentrations from 12.5 to 200 mM. We determined the distribution of each acceptor product and the amount of dextran synthesized for each of these described conditions. The data show how the amount of dextran and acceptor-products can be controlled and obtained in different proportions, and how the first acceptor-product can be exclusively obtained in maximum yield with very little or no dextran being formed.

EXPERIMENTAL

Carbohydrates and reagents.—[U-^{14}C]Sucrose and maltose were purchased from Sigma Chemical Co. (St. Louis, MO). Whatman K5 TLC plates were purchased from Fisher Scientific (Chicago, IL). All other chemicals were of reagent grade and commercially available.

Enzyme.—*Leuconostoc mesenteroides* B-512FM dextransucrase was purified according to the method of Fu and Robyt[7]. Enzyme activity was determined by a radioactive assay using [^{14}C]sucrose and is given in International Units (IU)[7], i.e., μmol of D-glucose from sucrose incorporated into dextran per min under the optimum conditions of pH 5.2 and 25°C.

Acceptor-reaction digests.—The acceptor reaction digests all were 100 μL and contained various proportions of [U-^{14}C]sucrose (1 μCi) and maltose, 20 mM

pyridine–acetic acid buffer (pH 5.2), and 12–1.2 × 10^4 mIU of dextransucrase. For the study of the effects of the ratio of maltose to sucrose and the effect of different concentrations of maltose and sucrose, 120 mIU/100 μL of digest was used. The reactions were conducted for 24 h (several conversion periods) at 25°C. Aliquots (5 μL) were removed and spotted onto Whatman K5 TLC plates for chromatography with 3 ascents 2:5:3 of $MeNO_2$–1-propanol–water. After development, the plates were exposed to X-ray film for 15 h and then the labeled compounds that were separated on the plate were quantitatively determined by using an autoscanning PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Effect of different ratios of maltose to sucrose using 100 mM constant amount of carbohydrate.—Fig. 1 and Table I show the number, kind, and quantitative amounts of the products formed when the ratio of maltose to sucrose (M/S) was varied from 0 to 100:1, using a constant concentration (100 mM) of total carbohydrate in the digest. As the M/S ratio was increased, the amount of dextran

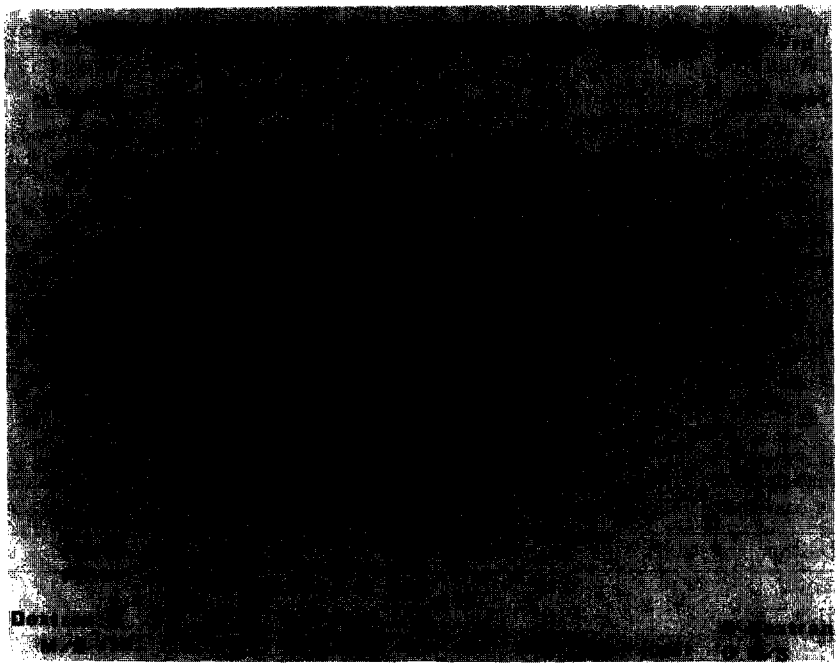

Fig 1. Thin-layer chromatographic autoradiogram of [^{14}C]sucrose acceptor reactions using different ratios of maltose to sucrose at 100 mM constant total carbohydrate and 120 mIU/100 μL. The chromatography was conducted on Whatman K5 plates using 3 ascents of 2:5:3 nitromethane–1-propanol–water. Pn is a saccharide acceptor-product of dp = *n*, having an isomaltodextrin chain linked α-(1 → 6) to the nonreducing end of maltose; Fru = D-fructose, Lec = leucrose.

TABLE I

Relative weight percent of dextran and acceptor-products [a] formed as a function of the ratio of maltose to sucrose at a constant carbohydrate concentration of 100 mM and 120 mIU of dextransucrase per 100 μL

M/S [b]	Dextran	P3	P4	P5	P6	P7	P8	P9	P10 [c]
0	100.0								
1:5	47.9	9.6	10.6	12.8	9.1	4.1	2.8	1.7	1.4
1:3	36.5	9.7	14.7	16.3	10.3	5.5	3.5	2.2	1.3
1:1	14.1	38.9	30.5	12.7	2.9	0.7	0.2		
2:1	7.7	59.4	26.5	5.6	0.7	0.2	0.1		
4:1	4.6	74.0	19.1	2.2	0.1				
5:1	4.4	76.8	17.1	1.7					
10:1	2.2	87.7	9.6	0.5					
20:1	0.3	95.0	3.0	0.1					
40:1	0.1	96.8	3.0	0.1					
100:1	0.0	98.7	1.3						

[a] The percentages do not include D-glucose formed by hydrolysis (acceptor reaction with water) or D-glucose incorporated into leucrose (acceptor-product of D-fructopyranose). [b] M/S, the ratio of the concentrations of maltose to sucrose. [c] Pn represents a saccharide acceptor-product of dp = n, having an isomaltodextrin chain linked α-(1 → 6) to the nonreducing end of maltose.

decreased and the amount of acceptor products was concomitantly increased, although the *number* of acceptor products was decreased. A ratio of 1:5 gave 47.9% dextran and 52.1% in ten acceptor-products. When the M/S ratio was 5:1, 4.4% dextran was formed and 95.6% in three acceptor-products: panose, a trisaccharide (P3); a tetrasaccharide, 6^2-*O*-α-isomaltosylmaltose (P4), and a pentasaccharide, 6^2-*O*-α-isomaltotriosylmaltose (P5) were formed. When the ratio was 100:1, no dextran was formed and only two acceptor products, 98.7% P3 and 1.3% P4 were formed.

Effect of different ratios of maltose to sucrose using 50 mM sucrose and varying concentrations of maltose.—The results of these conditions are given in Table II. The amount of dextran rapidly decreased and the amount of acceptor-products

TABLE II

Relative weight percent of dextran and acceptor-products [a] formed as a function of the ratio of maltose to sucrose at a constant concentration of 50 mM sucrose and 120 mIU of dextransucrase per 100 μL

M/S [b]	Dextran	P3	P4	P5	P6	P7	P8	P9	P10 [c]
1:5	66.6	4.1	8.1	9.7	6.4	2.9	1.5	0.7	
1:3	51.8	8.0	14.2	14.8	7.3	2.5	1.0	0.4	
1:1	14.1	38.9	30.5	12.7	2.9	0.7	0.2		
2:1	7.5	55.0	30.0	6.1	1.4				
4:1	4.2	72.8	20.8	2.1	0.1				
5:1	2.8	79.8	17.4						
10:1	0.8	90.8	8.4						
20:1	0.0	97.4	2.6						

[a,b,c] See footnotes to Table I.

TABLE III

Relative weight percent of dextran and acceptor-products [a] formed as a function of the ratio of maltose to sucrose at a constant concentration of 100 mM sucrose and 120 mIU of dextransucrase per 100 μL

M/S [b]	Dextran	P3	P4	P5	P6	P7	P8	P9	P10 [c]
1:5	47.0	5.7	7.8	11.7	11.0	6.8	5.1	2.9	2.0
1:3	32.0	8.2	14.8	19.2	12.9	6.1	3.7	2.0	1.1
1:1	6.5	40.3	32.0	15.7	4.5	0.8	0.2		
2:1	3.8	55.2	32.6	7.3	1.1				
4:1	1.5	75.4	20.5	2.0	0.6				
5:1	1.1	80.1	17.4	1.4					
10:1	0.1	91.8	8.1						
20:1	0.0	100.0							

[a,b,c] See footnotes to Table I.

increased as the ratio was increased. At a ratio of 1:5, 66.6% dextran was formed and there were seven acceptor-products. As the ratio was increased to 5:1, only 2.8% dextran was formed and there were only two acceptor products: 79.8% panose (P3) and 17.4% 6^2-*O*-α-isomaltosylmaltose (P4). At a ratio of 20:1, no dextran was formed and 97.4% panose and 2.6% tetrasaccharide (P4) were formed.

Effect of different ratios of maltose to sucrose using 100 mM sucrose and varying concentrations of maltose.—The results of these sets of conditions are given in Table III. When the concentration of sucrose was doubled to 100 mM, the amount of dextran formed decreased more rapidly than the 50 mM digest as the ratio of maltose to sucrose was increased. At a ratio of 1:5, 47.0% dextran was formed and there were eight acceptor-products. When the ratio was increased to 5:1, only 1.1% dextran was formed with three acceptor-products: 80.1% panose (P3), 17.4% (P4), and 1.4% (P5). When the ratio was increased to 20:1, no dextran was formed and there was only one acceptor-product, panose (P3).

The maximum yields of the individual acceptor products changed as the M/S ratio was changed: P3 was maximum at 20:1, P4 at 2:1, and P5 and P6 at 1:3.

Effect of substrate concentration using a 1:1 ratio of maltose to sucrose.—Table IV shows the effect of increasing the concentrations of maltose and sucrose from 1.25 to 300 mM while keeping the M/S ratio constant at 1:1. The major effect of increasing the concentration of the substrates is the decrease in the amount of dextran formed. The amount of panose (P3) did not change drastically, only varying between 34 and 41%. The amount of P4 increased steadily with increasing concentrations of the substrate, although not changing drastically after the concentrations reached 50 mM. This data indicates that the concentrations of the substrates have the most significant effects on the amount of dextran formed and that the ratio of acceptor to sucrose (Tables I–III) have significant effects on the amounts and number of individual acceptor products formed.

Effect of enzyme concentration.—Figs. 2 and 3 and Table V show the effects of the amount of enzyme and the concentration of substrates at a ratio of 1:1.

TABLE IV

Relative weight percent of dextran and acceptor-products [a] formed as a function of a 1:1 ratio of different concentrations of maltose and sucrose and 120 mIU dextransucrase per 100 μL

M/S [b] (mM/mM)	Dextran	P3	P4	P5	P6	P7	P8 [c]
1.25:1.25	49.3	34.3	13.4	2.8	0.1		
5:5	32.5	41.0	18.9	5.5	1.3	0.7	
12.5:12.5	24.0	42.9	22.4	7.4	3.0	0.3	
25:25	20.0	40.0	25.7	10.6	2.8	0.7	0.2
50:50	14.1	38.9	30.5	12.7	2.9	0.7	0.2
100:100	6.5	40.2	31.7	15.8	4.5	0.9	0.2
150:150	4.5	39.2	33.7	16.7	4.8	1.0	0.1
200:200	2.6	40.0	34.0	16.9	4.8	1.3	0.4
250:250	1.4	40.0	36.0	16.6	5.7	1.3	
300:300	0.6	41.2	37.1	16.2	4.4	0.6	

[a,c] See footnotes to Table I.

Enzyme amounts of 12 mIU to 1.2×10^4 mIU per 100 μL of digest were used with four 1:1 maltose and sucrose concentrations of 12.5 to 200 mM. Fig. 2 and Table V show that the amount of dextran decreased and the amounts of acceptor-products increased as the amount of enzyme was increased. This was most apparent at the lower concentrations of substrate (12.5 and 50 mM). At the higher concentrations of substrate (100 and 200 mM), the amount of dextran was low at all concentrations of enzyme. The highest concentration of enzyme (12000 mIU/100 μL) gave 96–99% acceptor-products for all concentrations of maltose and sucrose. Further, Fig. 3 shows that the number of acceptor-products increased as the

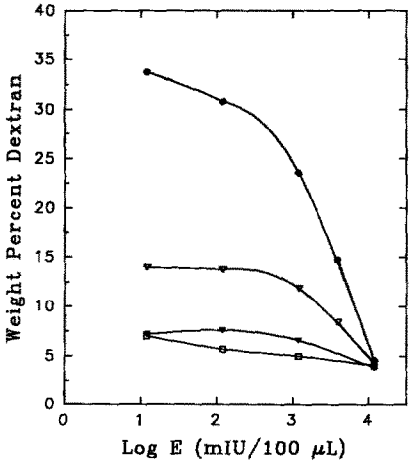

Fig. 2. Effect of different concentrations of enzyme on the amount of dextran synthesized from sucrose, using 1:1 ratios of different concentrations (12.5–200 mM) of maltose and sucrose. ●, 12.5; ▽, 50; ▼, 100; □, 200 mM. E, the concentration of enzyme, is expressed as mIU/100 μL of digest.

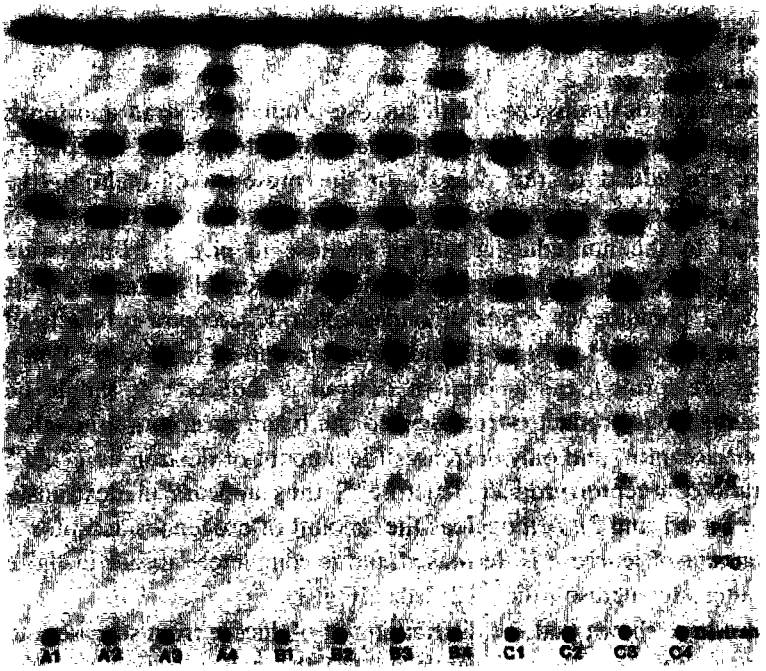

Fig. 3. Thin-layer chromatogram of [^{14}C]sucrose reactions using a 1:1 ratio of different concntrations of maltose and sucrose and different amounts of dextransucrase. A, 12.5; B, 50; C, 100 mM maltose and sucrose; the numbers 1, 2, 3, and 4 with A, B, and C represent 12, 120, 1200, and 12000 mIU/100 μL of digest, respectively. Fru = fructose, Leu = leucrose, Pn is a saccharide acceptor-product of dp = n, having an isomaltodextrin chain linked α-(1 → 6) to the nonreducing end of maltose.

amount of enzyme was increased. As the amount of enzyme was decreased to 12 mIU/100 μL, the number of acceptor-products was decreased (see Lanes A1, B1, and C1 of Fig. 3 and Table V). Lanes A4 and B4 of Fig. 3 showed some secondary acceptor-products that were not present in the other digests and were not identified. At the highest concentrations of enzyme, lanes A4, B4, and C4 also showed an increase in the D-fructose acceptor-product, leucrose. Lane A4 also showed the secondary D-fructose acceptor-products, isomaltulose and 6^2-O-α-glucopyranosylmaltulose. Lanes A3, B3, and C3 also showed leucrose but of a lesser amount. The major products in all of the digests were the homologous series of saccharides (P3–P10), having isomaltodextrins attached α-(1 → 6) to the nonreducing end of the maltose acceptor[5,6].

Table V shows that at maltose and sucrose concentrations of 50 and 100 mM, the amount of P3 and P4 remained relatively constant as the concentration of the enzyme was increased. The amounts of P3 and P4 also remained relatively constant when the substrate concentrations were 12.5 mM and the concentration of enzyme was increased, with the exception for the highest concentration of enzyme in which there was an 18% increase in P3.

DISCUSSION

The usual reaction of dextransucrase with sucrose produces dextran and forms D-fructose. This reaction is altered when other carbohydrates (called acceptors), such as maltose, are added to the digest[5]. In the presence of maltose, the D-glucose moiety of sucrose is diverted from the synthesis of dextran and is transferred instead to the nonreducing end of maltose to give a trisaccharide acceptor-product, panose[6]. The D-glucose can also be transferred to the acceptor-product(s) to give a homologous series of oligosaccharide acceptor-products of isomaltodextrins attached α-(1 → 6) to the nonreducing end of maltose[5,6]. When acceptor-products are formed, the amount of dextran is reduced[6]. It should be noted that the endogenous product D-fructose also can be an acceptor, although it is a relatively poor acceptor, and only decreases the amount of dextran by 1–2%[6]. The D-fructopyranose acceptor-product, leucrose[5,8], thus appears in dextransucrase digests (see Figs. 1 and 3). Although as the amount of exogenous acceptor is increased, the amount of leucrose is decreased and is completely absent in those digests with a maltose to sucrose ratio $> 2:1$ (see Fig. 1).

In this study, we have shown that the distribution of D-glucose from sucrose into dextran and into acceptor-products depends on (*a*) the concentration ratio of acceptor to sucrose, (*b*) the concentration of sucrose, and (*c*) the amount of enzyme present in the digest.

In general, as the ratio of acceptor to sucrose increases, the amount of dextran

TABLE V

Relative weight percent of dextran and acceptor products [a] formed as a function of the amount of enzyme and the concentration of maltose and sucrose

Enzyme (mIU/100 μL)	Dextran	P3	P4	P5	P6	P7	P8	P9	P10 [c]
12.5 mM Maltose and sucrose [b]									
12	33.7	38.8	22.2	4.8	0.5				
120	30.8	37.5	23.3	7.0	1.2	0.2			
1200	23.6	37.1	21.8	11.0	4.4	1.5	0.6		
12000	4.6	55.5	22.8	10.5	4.4	1.6	0.6		
50 mM Maltose and sucrose [b]									
12	14.0	39.7	35.0	9.6	1.5	0.2			
120	13.3	36.9	32.8	13.4	3.0	0.4	0.2		
1200	12.6	36.1	24.7	14.8	7.0	2.8	1.2	0.5	0.2
12000	4.3	41.6	25.3	14.5	7.2	3.7	2.0	1.0	0.4
100 mM Maltose and sucrose [b]									
12	7.2	38.4	39.2	12.4	2.4	0.4			
120	7.6	36.4	35.2	16.1	3.8	0.7	0.2		
1200	6.7	35.9	26.5	16.9	8.3	3.3	1.5	0.7	0.3
12000	3.8	39.2	25.1	15.5	8.6	3.8	2.1	1.2	0.6

[a,b,c] See footnotes to Table I.

synthesized decreases and the amount of acceptor-products increases. For acceptors such as maltose, that give a homologous series of acceptor products, the *number* of acceptor products also decrease as the ratio is increased. For a maltose acceptor reaction, in which the total amount of carbohydrate is kept constant at 100 mM, a ratio of 1:5 gave a series of ten homologous acceptor-products, and a ratio of 80:1 gave only one acceptor-product. In a similar reaction, but with a constant sucrose concentration of 100 mM, a maltose to sucrose ratio of 20:1 gave only a single acceptor-product, panose, with no dextran being formed.

This is in contrast to the condition of using relatively high maltose and sucrose concentrations in a ratio of 1:1, such as 300 mM, which also gave a high yield of acceptor-products (99.4%) and a low yield of dextran (0.6%). In this reaction, five homologous acceptor-products were formed. At a sucrose concentration of 300 mM, however, it would not be possible to have a maltose to sucrose ratio of 20:1, which would require a concentration of 6 M maltose, and certainly not 80:1, which would require a maltose concentration of 24 M.

The effect of the enzyme concentration on the product distribution was unexpected and not readily interpreted. Nevertheless, the effect is real. An increase in the amount of enzyme gave a decrease in the amount of dextran synthesized (Fig. 2) and an increase in the number and the amount of oligosaccharide acceptor-products (Table V and Fig. 3). This effect was dependent on the concentrations of sucrose and maltose. Differences in the amount of enzyme were more apparent at the lower concentrations of sucrose and maltose (12.5 and 50 mM) than at the higher concentrations (> 100 mM). At a concentration of 12.5 mM sucrose, the lowest amount of enzyme (12 mIU) gave 66.3% acceptor products, while the highest amount of enzyme (1.2×10^4 mIU) gave 95.4% acceptor-products. At a maltose and sucrose concentration of 200 mM, the lowest amount of enzyme gave 93.5% acceptor-products and the highest amount of enzyme gave 96% acceptor-products (see Fig. 2).

In 1955, Tsuchiya et al.[9] made a related observation in which they found that increasing the enzyme concentration decreased the amount of high-molecular-weight dextran. At the time, Tsuchiya et al. did not put forward an explanation. Our results would suggest that the explanation involves an increase in the acceptor reactions at the higher enzyme concentrations. It is known that higher enzyme concentrations would give an increase in the formation of D-glucose and D-fructose[10] due to an increase in the acceptor reaction with water (essentially the hydrolysis of sucrose). D-Glucose and D-fructose are acceptors. The acceptor-product from D-glucose is isomaltose. Isomaltose also is an acceptor that is more efficiently bound than D-glucose[3]. Then, there would be an increase in the amounts of low-molecular-weight isomaltodextrin acceptor-products as well as the early build up of D-fructose that can act as acceptors for the covalently linked dextran chain[5]. The dextran chains would then be displaced from the active site by the acceptors before the dextran chains become very large. Thus, at the higher enzyme concentrations, the increased amount of acceptor-products can act as

acceptors for dextran, terminating dextran synthesis by releasing it from the active site, giving a low-molecular-weight dextran.

In summary, the data of Tables IV and V show that although the concentrations of the substrates (acceptor and sucrose) and the concentration of enzyme have an effect on the amounts of dextran and acceptor-products formed, the data of Tables I–III indicate that the concentration ratio of acceptor to sucrose has a more profound effect on the amount of dextran and the amount and number of acceptor products formed. Thus, although the concentration of acceptor and sucrose and the concentration of enzyme must be considered, the ratio of the acceptor to sucrose is the more important factor to be considered in controlling the amount of dextran and the amount and number of acceptor-products synthesized by dextransucrase. A high yield of 4–6 acceptor-products can be obtained at any 1:1 acceptor to sucrose concentration by using a high concentration of enzyme. However, for the formation of a single acceptor-product (e.g., panose in this study), a concentration of 100 mM sucrose with a maltose to sucrose ratio of 20:1 and an enzyme concentration of 120 mIU/100 μL of digest could be used.

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