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Mechanism of the action of *Leuconostoc mesenteroides* B-512FMC dextransucrase: kinetics of the transfer of D-glucose to maltose and the effects of enzyme and substrate concentrations

Motomitsu Kitaoka [1], John F. Robyt *

Laboratory of Carbohydrate Chemistry and Enzymology, 4252 Molecular Biology Building, Iowa State University, Ames, IA 50011, USA

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Abstract

The kinetics of the reaction of *Leuconostoc mesenteroides* B-512FMC dextransucrase with sucrose were studied. This enzyme catalyzes the synthesis of dextran from sucrose with a k_{cat} of 641 s^{-1} and the transfer of D-glucose from sucrose to maltose with a k_{cat} of 1070 s^{-1}. The enzyme was also found to catalyze two new reactions in the absence of sucrose, using dextran as the substrate; D-glucose was transferred from the non-reducing ends of dextran chains to maltose with a relatively low k_{cat} of 3.2 s^{-1}; and D-glucose was hydrolyzed from the non-reducing ends of dextran chains with a very low k_{cat} of 0.085 s^{-1}. Ping-pong/bi-bi kinetics of these reactions are consistent with the formation of a glucosyl–enzyme covalent intermediate. It is shown that an increase in the concentrations of both maltose and sucrose in the D-glucose transfer reaction to maltose gives an exponential decrease in the amount of dextran and a concomitant increase in the amount of acceptor products. It is further shown that increasing the amount of dextransucrase gives a decrease in the amount of dextran and an increase in the amount of acceptor products, after the sucrose has been consumed. This anomaly occurs because the relatively high amounts of enzyme catalyze the transfer of D-glucose from the non-reducing ends of the dextran chains to maltose, giving a decrease in the amount of dextran and an increase in the amount of acceptor product. Further, the high amounts of enzyme catalyze the hydrolysis of the D-glucose residues from the ends of the dextran chains, giving a decrease in the amount of dextran. These reactions are not observed when lower amounts of enzyme are used, as the reactions are much slower than the synthesis of dextran and the usual acceptor transfer reactions of D-glucose from sucrose to acceptor.

Keywords: Dextransucrase; Maltose acceptor reactions; Transfer reactions; Hydrolytic reactions; Effects of substrate concentrations; Effects of enzyme concentration; Kinetics; *Leuconostoc mesenteroides* B-512FMC

1. Introduction

It is generally recognized that dextransucrase catalyzes three reactions, using sucrose as the substrate: (1) the synthesis of dextran, (2) the synthesis of dextran branch linkages, and (3) in the presence of other carbohydrates, such as D-glucose, D-fructose, maltose, etc.,

* Corresponding author. Tel.: +1-515-294-6116; fax: +1-515-294-0453.
E-mail address: jrobyt@iastate.edu (J.F. Robyt)
[1] Present address: National Food Research Institute, Kannondai, Tsukuba, Ibaraki 305, Japan.

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the transfer of D-glucose from sucrose to the carbohydrate (reviewed in Ref. [1]). The latter two reactions are commonly called *acceptor reactions*.

The mechanism of the synthesis of dextran has been shown to involve a two-site insertion mechanism in which D-glucose and the growing dextran chain are covalently attached to the active site. The D-glucose is transferred to the reducing end of the growing dextran chain [1,2]. The branch linkages by the action of the synthetic enzyme (dextransucrase) form when a dextran chain acts as an acceptor and displaces either the D-glucose or the dextran chain from the active site, forming α-(1→3) branch linkages [1,3].

Leuconostoc mesenteroides B-512F dextransucrase can transfer D-glucose from sucrose to several different carbohydrate acceptors [1,4]. Maltose is known to be the most effective acceptor [4], forming panose [6^2-α-D-glucopyranosyl maltose], which also can be an acceptor to give a tetrasaccharide, which also can be an acceptor to eventually give a homologous series of 6^2-α-isomaltodextrinyl maltose products [4,5]. Su and Robyt [5] studied the effects of the ratio of maltose to sucrose (M/S) on the acceptor reaction and found that the ratio significantly affected the amount and number of products formed. When the ratio was low (0.2), about 50% of the D-glucose available from sucrose was incorporated into dextran and the remaining 50% was incorporated into eight maltose acceptor-products. When the maltose to sucrose ratio was high, for example when M/S = 100 (100 mM maltose + 1 mM sucrose) and when M/S = 20 (2 M maltose + 100 mM sucrose), no dextran was formed and only a single trisaccharide product, panose, was formed [5]. Su and Robyt [5] also found that the distribution of D-glucose into dextran and into the acceptor products was dependent on the concentrations of sucrose and the acceptor. Further, they found that the increase in the amount of enzyme caused a decrease in the amount of dextran and a concomitant increase in the amount of acceptor products. These two observations were relatively unusual and not readily explained. In the present study, the kinetics of the maltose acceptor reaction were studied, along with the effects of the substrate- and the enzyme concentrations on the acceptor reaction in an attempt to understand the effects of substrate and enzyme concentrations on the reactions.

2. Experimental

Materials

Enzyme. Dextransucrase was prepared by growing a high-producing constitutive mutant of *L. mesenteroides* B512FMC-16 [6] in a D-glucose medium and purifying it as previously described [7]. The enzyme used in this report was electrophoretically pure on PAGE, with a molecular mass of 190 kDa. The dextransucrase activity was measured using [*U*-^{14}C]sucrose as previously described [6]. One international unit (IU) of dextransucrase activity is defined as the amount of enzyme that incorporates 1 μmole of ^{14}C-D-glucose into dextran from 125 mM [*U*-^{14}C]sucrose at 21 °C. The specific activity of dextransucrase was 183 IU mg^{-1}.

Dextrans. B-512F-Dextran T-10, T-40, and T-500 were obtained from Pharmacia Biotech Inc. (Piscataway, NJ). The properties of the dextrans are summarized in Table 1.

Methods

Inhibition of the synthesis of dextran by maltose. All dextransucrase reactions contained 1 mg mL^{-1} Tween 80 and 1 mM $CaCl_2$ to give maximum activity and enzyme stability [8,9].

A series of reactions was carried out in 200 μL digests containing 1.3 μCi mL^{-1} [*U*-^{14}C]-sucrose at concentrations of 5, 10, 20, 30, or 50 mM and maltose concentrations of 0, 20, or 40 mM. The reactions were initiated by the addition of 0.05 IU of dextransucrase per mL, which was in 10 mM NaOAc buffer (pH 5.2) containing 1 mg mL^{-1} Tween 80 and 1 mM $CaCl_2$ at 21 °C. The amount of radioactivity incorporated into dextran was measured every 10 min for 40 min by taking 25 μL aliquots and adding them to 1.5 × 1.5 cm Whatman 3MM filter papers, which were immediately dropped into MeOH; the papers were washed twice with MeOH, and the amount of ^{14}C present on the paper was determined in a toluene cocktail by liquid scintillation spectrometry.

Effect of the enzyme concentration on the maltose acceptor reaction. The maltose acceptor reaction, conducted by using different amounts of enzyme, was performed in 400 μL digests containing 5 μCi mL^{-1} [*U*-^{14}C]sucrose (12.5 or 50 mM) and dextransucrase (42, 4.2 or 0.42 IU mL^{-1}) in 10 mM NaOAc buffer (pH 5.2), containing 1 g L^{-1} Tween 80 and 1 mM $CaCl_2$ at 21 °C. Aliquots (25 μL) of the digests were added to 5 μL of 60% (v/v) pyridine at various times to make the pH 10 and stop the reaction. Then, 10 μL of the solution was spotted onto a Whatman K6F TLC plate (20 × 20 cm), followed by three ascents with 7:3 (v/v) MeCN–water, path-length of 18.5 cm. The labeled compounds, D-fructose, D-glucose, sucrose, acceptor products, and dextran were quantitatively determined on the TLC plate by using a PhosphoImager (Molecular Dynamics, Sunnyvale, CA).

Assay of the D-glucosyl transfer-reaction from sucrose and from dextran to maltose. D-Glucosyl transfer reaction from sucrose to maltose was performed at concentrations of 10, 20, 40, and 100 mM sucrose and 20, 40, and 80 mM maltose in 10 mM NaOAc buffer (pH 5.2), containing 1 mg mL^{-1} Tween 80 and 1 mM $CaCl_2$ with 0.125 IU mL^{-1} dextransucrase at 21 °C.

The D-glucosyl transfer reaction from dextran to maltose was studied, using three kinds of dextrans (dextran T-10, T-40, and T-500) at concentrations of 1, 2, 5, 10 mg mL^{-1} and 50 mM maltose in 10 mM NaOAc buffer (pH 5.2), containing 1 mg mL^{-1} Tween 80 and 1 mM $CaCl_2$ with 12.5 IU mL^{-1} dextransucrase at 21 °C. The kinetic constants of each dextran as a glucosyl donor were determined. The mechanism of the reaction was further determined by using various concentrations of 1, 2, 4, and 10 mg mL^{-1} dextran T-500 with 20, 40, and 80 mM maltose.

To measure the increase in the acceptor product (panose), 50 μL-aliquots of the digests were taken every 10 min for 40 min and added to 50 μL of 10% (v/v) pyridine (pH 9) to terminate the reaction. The solution was then diluted four-fold with water and 1 μL of the diluted solution was put onto a Whatman K5F TLC plate (20 cm width × 10 cm length). The TLC plate was then irrigated four times with 85:15 (v/v) MeCN–water using a path-length of 8.5 cm. Carbohydrates on the TLC plate were detected by dipping the plate into MeOH containing 0.3% (w/v) *N*-(1-naph

Table 1
Characteristics and kinetic constants for the dextrans used to study glucosyl transfer to 50 mM maltose (forming panose) and the hydrolysis (forming D-glucose)

Dextran characteristics	Dextrans T-10	T-40	T-500
M_w [a]	9,700	42,500	465,000
M_n [a]	6,000	26,500	191,500
Degree of branching (DB) [a,b]	5%	5%	5%
Reducing ends [c]	0.17	0.038	0.005
Non-reducing ends [c]	0.48	0.35	0.30
Kinetic constants for the glucosyl transfer from dextran chain to maltose			
	Dextrans T-10	T-40	T-500
K_{mapp} (g L^{-1})	0.97 ± 0.16	0.53 ± 0.11	0.96 ± 0.12
k_{catapp} (s^{-1})	2.40 ± 0.10	1.90 ± 0.10	2.00 ± 0.10
Kinetic constants for hydrolysis of glucosyl residues from the non-reducing ends of dextran chains			
	Dextrans T-10	T-40	T-500
K_m (mg mL^{-1})	47 ± 2	27 ± 1	19 ± 2
K_{cat} (s^{-1})	0.086 ± 0.002	0.090 ± 0.002	0.085 ± 0.002

[a] The number-average molecular weight, the weight-average molecular weight (M_n and M_w) and the degree of branching (DB) were obtained from the technical bulletin provided by Pharmacia.

[b] Degree of branching is defined as (branching glucose unit)/(total glucose unit) × 100.

[c] The numbers of reducing ends and non-reducing ends are given in mmol g^{-1} of dextran and were calculated using the following relationships: reducing ends = $1000/M_n$; non-reducing ends = $[(1000M_n/162)(DB/100)+1000]/M_n$.

thyl)ethylendiamine and 5% (v/v) H_2SO_4, followed by heating the plate at 120 °C for 20 min [10]. The increase in the acceptor product, panose, in the early stages of the reaction was measured on the TLC plate by using a densitometer (model GS-670, BioRad, Hercules, CA) from which the initial rate of the acceptor reaction was determined [11].

Assay of dextran hydrolysis. Hydrolysis of dextran by dextransucrase was studied at four concentrations of dextran (0.05, 0.1, 0.2, and 0.5 mg mL^{-1}) using three kinds of dextran (T-10, T-40 and T-500) in 10 mM NaOAc buffer (pH 5.2), containing 1 mg mL^{-1} Tween 80 and 1 mM $CaCl_2$ with 3.1 IU mL^{-1} dextransucrase at 21 °C. Aliquots (600 μL) were taken every 30 min for a total of 120 min and the reaction was stopped by the addition of 140 μL of 0.2 M NaOH containing 0.1 M Tris–HCl. After 10 min, the digest was neutralized by adding 110 μL of 0.2 M HCl. A 200 μL-portion of the neutralized solution was then put into the well of a microtiter plate and the amount of glucose determined using glucose oxidase [12].

Determination of kinetic constants. The kinetic equations were the standard Lineweaver–Burk equations for one and two substrate reactions and for competitive, noncompetitive, and mixed inhibition reactions [13]. Kinetic constants (K_m and k_{cat}) were obtained by plotting the data $1/v_i$ versus 1/[S] and by using a curve-fitting method of least-squares regression analysis [14].

3. Results

Maltose inhibition of dextran synthesis.— The Lineweaver–Burk plots of $1/v_i$ versus 1/[Suc] for the formation of dextran in the presence and absence of maltose are shown in Fig. 1. From the types of lines obtained, the inhibition pattern was determined to be mixed-type inhibition. The kinetic constants that were determined are given in Table 2. The K_m^{Suc} was 13.2 mM, very close to that previously reported (9 mM) [15].

Time course of the maltose acceptor reaction.—Fig. 2(A) illustrates the time course of the transfer of D-glucose from sucrose to maltose at sucrose and maltose concentrations of 12.5 mM each; Fig. 2(B) is the time course for sucrose and maltose concentrations of 50 mM each. The decrease in the amount of dextran with the concomitant increase in the amount of acceptor products is apparent in the first 200 min of the reaction, especially for the low concentrations of the substrates. There was also an increase in the DP of the acceptor products in the early stages (200 min), followed by a decrease in the DP in the later stage (after 500 min). This was especially apparent in the reaction with the higher amount (42 IU mL^{-1}) of enzyme with both the 12.5 mM (Fig. 2(A)) and the 50 mM (Fig. 2(B)) concentrations of the substrates. The increase in the amount of acceptor products with the decrease in the amount of dextran in the early stages of the reaction suggests the possibility that D-glucose residues of dextran were being transferred to the acceptor. The decrease in both dextran and the acceptor in the later stages, especially for the 12.5 mM concentration of substrates and high concentration of the enzyme (Fig. 2(A)) suggests the possibility that both dextran and the acceptor products were being hydrolyzed.

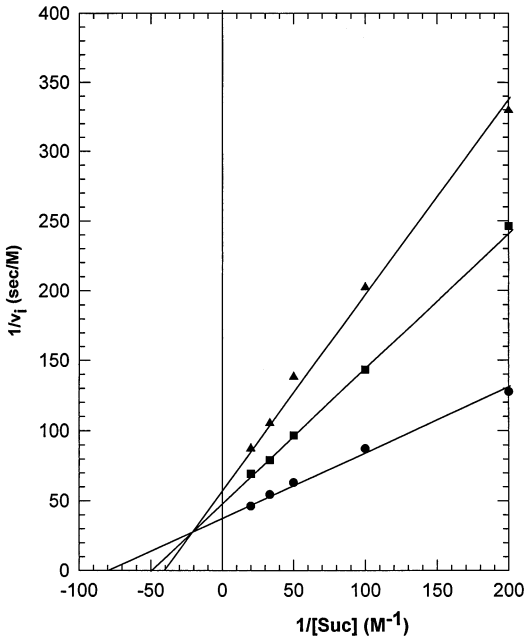

Fig. 1. Lineweaver–Burk plot of the formation of dextran in the absence and presence of maltose. Symbols (●, ■, ▲) represent the reaction with 0, 20, and 40 mM maltose, respectively.

Kinetic analysis of the transfer of D-*glucose from sucrose to maltose.*—Panose was the only acceptor product formed in the early (initial velocity) stages of the transfer reaction of D-glucose from sucrose to maltose. The Lineweaver–Burk plot, $1/v_i$ versus 1/[Suc], for the formation of panose is shown in Fig. 3. The parallel lines obtained for this plot are consistent for a ping-pong/bi-bi mechanism. The kinetic parameters for this reaction are given in Table 2. The k_{cat} value of the acceptor reaction is 1.67 times larger than that of the dextran-forming reaction. The K_m^{Suc} value (13.2 mM) is nearly identical to that observed for the formation of dextran (9 mM).

Kinetic analysis of the transfer of D-*glucose from dextran to maltose.*—In this experiment, only maltose and dextran were incubated together with dextransucrase. Panose was the only product observed in the early stages of the reaction. The kinetic constants (K_m^{Dex} and k_{cat}^{Dex}) for the three molecular sizes of dextran with 50 mM maltose are given in Table 1. The data show that the kinetic constants were not significantly affected by differences in the molecular weights of the dextrans.

The mechanism of the reaction of dextran with maltose was further studied, using B-512F-dextran T-500. The Lineweaver–Burk plot, $1/v_i$ versus 1/[dex], for the reaction is shown in Fig. 4. The parallel lines of this plot are consistent with a ping-pong/bi-bi mechanism. The kinetic parameters are given in Table 2. The K_m^{Mal} (29.2 mM) is essentially identical to the K_m^{Mal} (31.4 mM) obtained for the transfer of D-glucose from sucrose to maltose. The k_{cat}, however, is very low, 0.5% of the k_{cat} for the formation of dextran from sucrose and 0.3% of the k_{cat} for the formation of panose from the transfer of D-glucose from sucrose to maltose. These results suggest that because maltose has the same K_m for the two reactions, maltose is binding at the same binding site (the acceptor binding site) in the two reactions. The low k_{cat} for the transfer of D-glucose from the dextran chain to maltose indicates that the formation of the glucosyl–enzyme covalent complex by the transfer of D-glucose from dextran to the enzyme is a poor reaction in contrast to the transfer of D-glucose from sucrose to maltose.

Dextransucrase hydrolysis of dextran.—D-Glucose is the only hydrolysis product in the early stage of the reaction. Kinetic parameters of the hydrolysis of the three dextrans are summarized in Table 1. Neither K_m^{Dex} nor k_{cat}^{Dex} was significantly affected by the molecular weights of the dextrans. The k_{cat}^{Dex} values of the hydrolysis reaction are very low, between 0.013 and 0.014% of that of the k_{cat}^{Suc} for the synthesis of dextran. The K_m^{Dex} value of dextran hydrolysis reaction is significantly lower than the K_m^{Suc} obtained in the transfer reaction of D-glucose from sucrose to maltose.

Table 2
Kinetic constants involved in the maltose acceptor reactions and dextran inhibition and hydrolysis reactions

Synthesis of dextran with maltose as an inhibitor [a]	
K_m^{Suc}	13.2 ± 0.8 mM
$K_{i,s}$	21.1 ± 2.2 mM
$K_{i,i}$	65.3 ± 8.6 mM
k_{cat}	641 ± 14 s^{-1}
Glucosyl transfer from sucrose to maltose (formation of panose) [b]	
K_m^{Suc}	13.1 ± 2.0 mM
K_m^{Mal}	31.4 ± 4.5 mM
k_{cat}	1070 ± 62 s^{-1}
Glucosyl transfer from dextran T-500 to maltose (formation of panose) [c]	
K_m^{Dex}	1.57 ± 0.21 mg mL^{-1}
K_m^{Mal}	29.2 ± 5.4 mM
k_{cat}	3.2 ± 0.2 s^{-1}
Hydrolysis of dextran T-500 (formation of glucose)	
K_m	0.019 ± 0.002 mg mL^{-1}
k_{cat}	0.085 ± 0.002 s^{-1}

[a] The kinetic parameters for mixed inhibition appear in the following equation: $d[Dex]/dt = k_{cat}\,[E][Suc]/\{K_m^{Suc}(1+[Mal]/K_{i,s})+[Suc](1+[Mal]/K_{i,i})\}$.

[b] The kinetic parameters in ping-pong/bi-bi mechanism appear in the following equation: $d[Pan]/dt = k_{cat}[Suc][Mal]/([Suc][Mal]+K_m^{Suc}[Mal]+K_m^{Mal}[Suc])$.

[c] The kinetic parameters in ping-pong/bi-bi mechanism appear in the following equation: $d[Pan]/dt = k_{cat}[Dex][Mal]/([Dex][Mal]+K_m^{Dex}[Mal]+K_m^{Mal}[Dex])$.

4. Discussion

The Lineweaver–Burk plot for the synthesis of dextran from sucrose, in the presence of maltose, indicates that maltose is a mixed-type inhibitor (Fig. 1), which is essentially a non-

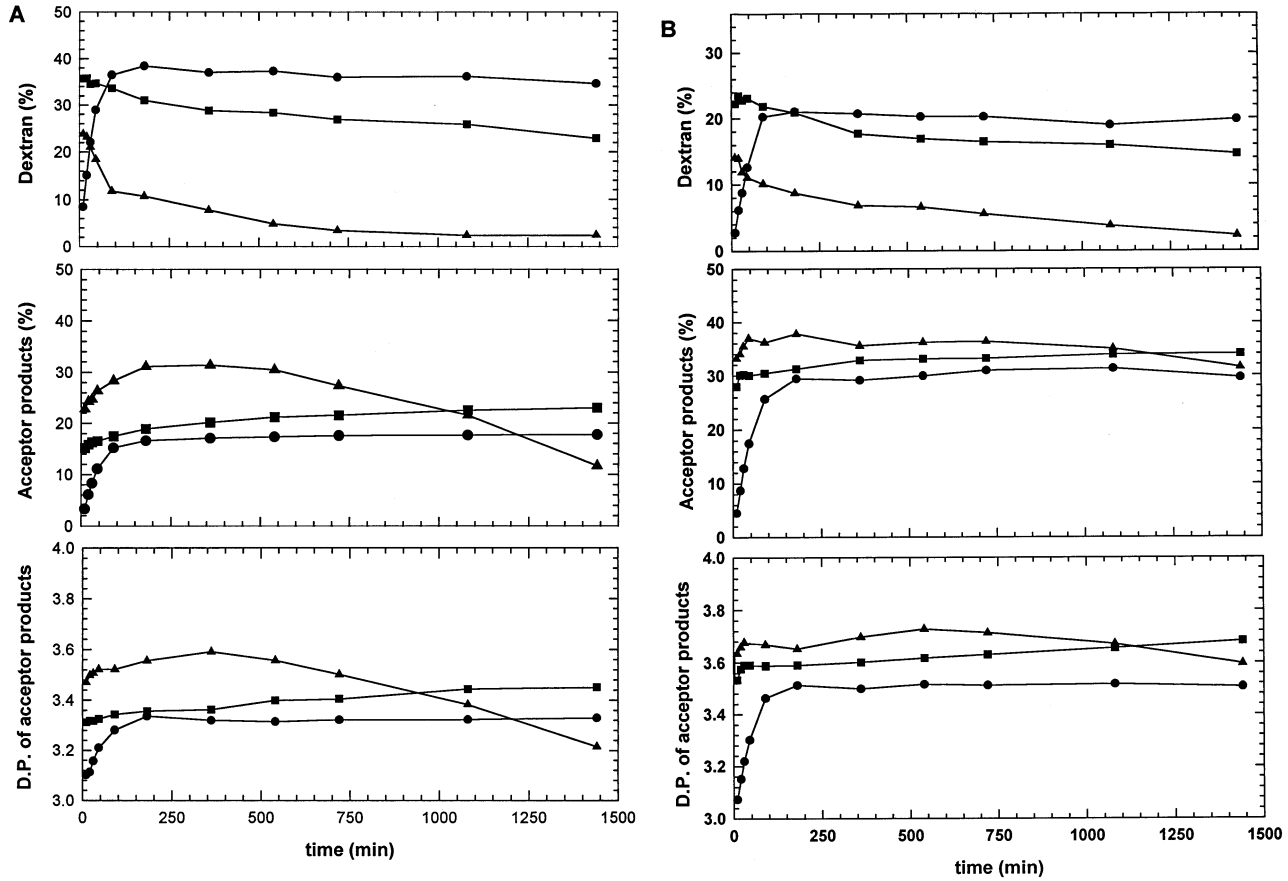

Fig. 2. Time course of the maltose acceptor reaction with (A) 12.5 mM sucrose and 12.5 mM maltose and with (B) 50 mM sucrose and 50 mM maltose; ●, ■, ▲ represent the reaction with 0.42, 4.2, and 42 IU mL^{-1} dextransucrase, respectively. The vertical axes of the top and the middle graphs represent the percentage of radioactivity incorporated into dextran and acceptor products, respectively. The vertical axis of the bottom graphs represents the average degree of polymerization of the acceptor products.

competitive inhibitor. This indicates that maltose (I) binds in the acceptor binding-site [16], forming an ESI complex in which the dissociation constant of sucrose (S) from ESI is different from the dissociation constant of sucrose from ES [15]. In this special kind of non-competitive inhibition, both K_m and k_{cat} are altered [17]. Maltose inhibits the synthesis of dextran by diverting the transfer of D-glucose away from dextran and to itself. The transfer of D-glucose from sucrose to maltose in the acceptor reaction gave parallel lines for the sucrose Lineweaver–Burk plot at three concentrations of maltose (Fig. 3), which is consistent with a ping-pong/bi-bi mechanism. This type of kinetics is characteristic of the formation of a covalent glucosyl–enzyme intermediate, which previously had been postulated by Robyt and co-workers [1–3,16] in the synthesis of dextran and in the formation of acceptor products. The k_{cat} value of the acceptor reaction from sucrose to maltose is 1.69 times larger than the k_{cat} value for the synthesis of dextran as shown in Table 2. This supports the observation that the rate of D-fructose released from sucrose is accelerated by the addition of some acceptors to the digest, as previously reported [18,19].

Su and Robyt [5] found that an increase in the equimolar concentration of sucrose and maltose gave a decrease in the ratio of dextran to acceptor product. The ratio of the initial rate of dextran formation versus the total activity was calculated from the data in Table 2 as the values of $d[Dex]/dt$ divided by $d[Dex]/dt + d[Pan]/dt$, and the percentage of D-glucose incorporated into dextran as a function of the equimolar concentration of sucrose and maltose was plotted as shown in Fig. 5. The plot of Fig. 5 indicates that the amount of D-glucose incorporated into dextran as a function of the concentration of sucrose and mal-

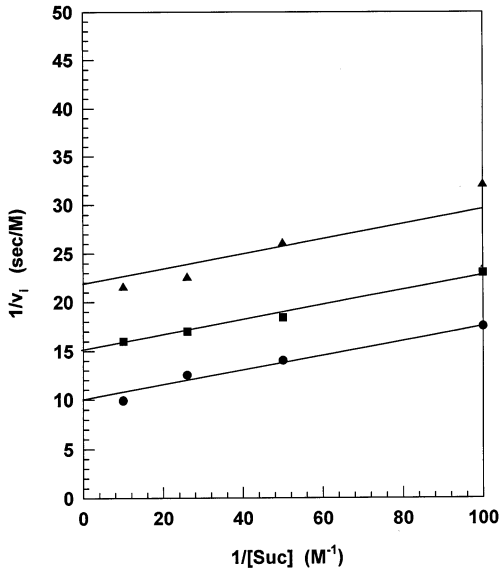

Fig. 3. Lineweaver–Burk plot of the dextransucrase-catalyzed formation of panose from sucrose and maltose; ●, ■, ▲, represent the reaction with 20, 40, and 80 mM maltose, respectively.

tose decreases exponentially and explains the substrate concentration effect on the dextran to acceptor products ratio as observed by Su and Robyt [5].

Su and Robyt [5] also reported that an increase in the amounts of dextransucrase produced a decrease in the amount of dextran and a concomitant increase in the amount of

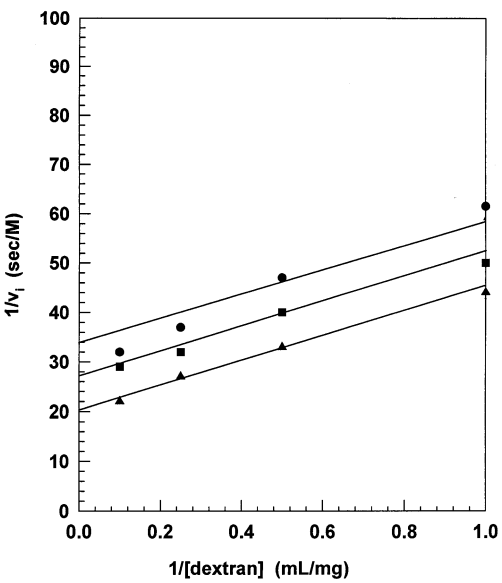

Fig. 4. Lineweaver–Burk plot of the dextransucrase-catalyzed formation of panose from dextran T-500 and maltose; ▲, ■, ● represent the reaction with 20, 40, and 80 mM maltose, respectively.

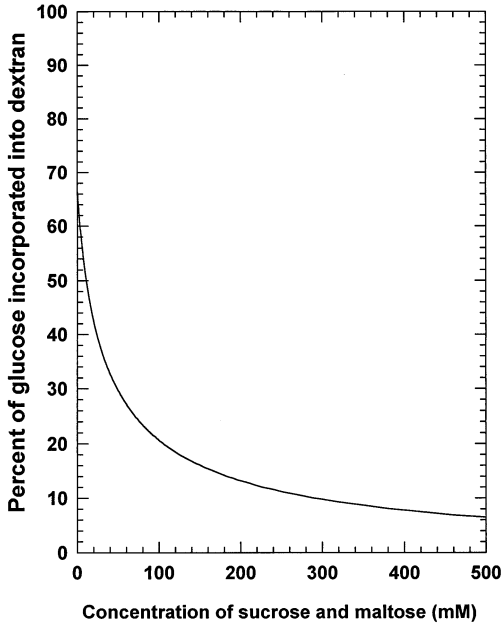

Fig. 5. Percentage of D-glucose incorporated into dextran as a function of the equimolar concentrations of sucrose and maltose. The curve was obtained using the kinetic constants shown in Table 2.

acceptor products when the 24 h, equilibrium amounts of products were measured. In the present report, the kinetics of the *initial stages* of these reactions, with various concentrations of dextransucrase, were studied instead of the late stage where equilibrium had occurred. The early stages (200 min) of the maltose acceptor reaction show that dextran decreased and the acceptor products increased after sucrose had disappeared (Fig. 2(A and B)). A possible explanation for these observations is that D-glucosyl units were being transferred from dextran to the acceptors. Such a transfer reaction had been previously reported by Binder and co-workers [20]. The decrease of the dextran and the concomitant increase in the amount of acceptor products could thus be due to the slow transfer of D-glucose from dextran to the acceptor(s). This reaction would especially be observed when the amount of enzyme is high but would not be as readily observed when the amount of enzyme is low, due to the relatively slow rate of transfer.

The study of the transfer of D-glucose from dextran to maltose was performed by using B-512F dextran T-500. It was found that the reaction was much slower than both the syn-

thesis of dextran from sucrose and the transfer reaction of D-glucose from sucrose to maltose. Transfer, however, did occur and panose was formed. The kinetics gave parallel lines (Fig. 4), indicating that the mechanism is also ping-pong/bi-bi and suggests the formation of the same covalent glucosyl–enzyme intermediate that was formed in both the maltose acceptor reaction and in the synthesis of dextran. Further, by using three B-512F dextrans of different molecular weights, no significant differences were observed in the kinetic constants of the transfer reaction (Table 1). This shows that the transfer was occurring from the non-reducing ends of the dextran chain because the number of non-reducing ends is not significantly affected by its molecular weight. If the transfer had been from the reducing end of dextran, the rates of reaction would have been inversely proportional to the molecular weights of the dextrans. The enzyme, thus, transfers D-glucosyl units from the non-reducing ends of dextran to maltose via a covalent glucosyl–enzyme intermediate.

The enzyme also catalyzes the hydrolysis of dextran, but at an extremely low rate. Because the K_m values for the hydrolysis did not significantly vary with the molecular weights of the dextrans, it is concluded that the enzyme hydrolyzes D-glucosyl residues from the non-reducing ends of dextran chains. The K_m value of dextran T-500 (meaning the apparent Michaelis constant for dextran [K^{Dex}_{mapp}] at $[H_2O] = 55$ M) was 0.019 mg mL^{-1}, a much smaller value than that obtained in the transfer reaction of D-glucose from dextran to maltose. From these results, the theoretical K_m value for H_2O ($K^{H_2O}_m$) in the hydrolysis is calculated to be 4490 M using the following ping-pong/bi-bi mechanism equation:

$$K^{Dex}_{mapp} = K^{Dex}_m\,[H_2O]/(K^{H_2O}_m + [H_2O])$$

When $K^{Dex}_{mapp} = 0.019$ mg mL^{-1}, $K^{Dex}_m = 1.57$ mg mL^{-1} (Table 2), and $[H_2O] = 55$ M, thus, showing that the enzyme has a very low affinity for water at its active site and therefore will catalyzes hydrolysis extremely slowly.

In summary, *L. mesenteroides* B-512F(MC16) dextransucrase catalyzes five different reactions. The first reaction synthesizes dextran from sucrose and forms α-(1→6) glycosidic bonds between D-glucose residues, with a k_{cat} of 641 s^{-1}. The second reaction forms α-(1→3) branch linkages by the transfer of glucosyl or dextranyl units from the active site to an exogenous dextran chain [3]. The third reaction, which occurs in the presence of sucrose and another carbohydrate, an acceptor (in this study, maltose), gives the transfer of D-glucose to maltose with a k_{cat} of 1070 s^{-1}. The fourth reaction, which occurs between dextran and maltose in the absence of sucrose, gives the transfer of D-glucose from the non-reducing ends of the dextran chains to maltose with a relatively low k_{cat} of 3.2 s^{-1}. The fifth reaction, which is exclusively with dextran, gives the extremely slow hydrolysis of D-glucose from the non-reducing ends of the dextran chains, with a very low k_{cat} of 0.085 s^{-1}.

The ping-pong/bi-bi kinetics that were observed for the reactions suggest that all of the reactions take place through the formation of a covalent glucosyl–enzyme intermediate. When the amount of enzyme is relatively high, the products from the fourth and fifth reactions are observed. Further, as the concentrations of the substrates in the third reaction (in this study, sucrose and maltose) are increased, there is an exponential decrease in the amount of dextran formed (Fig. 5). As previously reported [5], as the concentration ratio of acceptor to sucrose changes, there is a change in the amount of dextran formed and the amount of acceptor product formed. At low maltose to sucrose ratios, dextran and a homologous series of acceptor products are formed. As the acceptor to sucrose ratios are increased, there is a decrease in the amount of dextran formed and an increase in the amount of acceptor product formed but a decrease in the number of acceptor products in the homologous series. At some ratio, no dextran is formed and only a single acceptor product is formed.

The present results explain the previously unexplained observations [5] that the equilibrium amount of dextran formed to the equilibrium amount of acceptor product(s) formed decreases as the concentrations of sucrose and maltose increase and as the concentration of enzyme increases in the digest. The effect of the sucrose and maltose concentrations is due

to the more favorable k_{cat} for the acceptor reaction for maltose over the k_{cat} for the synthesis of dextran. The effects of increasing the amount of enzyme are not due to an impossible condition of a shift in the equilibrium of the reaction by the catalyst, but to the transfer of D-glucose from the non-reducing ends of the dextran chains to maltose when the amount of enzyme is increased to a relatively high level, and the decrease of dextran is further due to the hydrolysis of D-glucose residues from the non-reducing ends of the dextran chains when high amounts of enzyme are used.

References

[1] J.F. Robyt, *Adv. Carbohydr. Chem. Biochem.*, 51 (1995) 133–168.
[2] J.F. Robyt, B.K. Kimble, T.F. Walseth, *Arch. Biochem. Biophys.*, 165 (1974) 634–640.
[3] J.F. Robyt, H. Taniguchi, *Arch. Biochem. Biophys.*, 174 (1976) 129–135.
[4] J.F. Robyt, S.H. Eklund, *Carbohydr. Res.*, 121 (1983) 279–286.
[5] D. Su, J.F. Robyt, *Carbohydr. Res.*, 248 (1993) 339–348.
[6] M. Kitaoka, J.F. Robyt, *Enzyme Microb. Technol.*, 22 (1998) 527–531.
[7] M. Kitaoka, J.F. Robyt, *Enzyme Microb. Technol.*, 23 (1998) 386–391.
[8] A.W. Miller, J.F. Robyt, *Biochim. Biophys. Acta*, 785 (1986) 89–96.
[9] A.W. Miller, J.F. Robyt, *Biochim. Biophys. Acta*, 880 (1984) 32–39.
[10] R. Mukerjea, D. Kim, J.F. Robyt, *Carbohydr. Res.*, 292 (1996) 11–20.
[11] J.F. Robyt, R. Mukerjea, *Carbohydr. Res.*, 251 (1994) 187–202.
[12] J.D. Fox, J.F. Robyt, *Anal. Biochem.*, 195 (1991) 93–96.
[13] D.L. Purich, in D.L. Purich (Ed.), *Contemporary Enzyme Kinetics and Mechanisms*, Academic Press, New York, 1983, pp. 353–361.
[14] W.W. Cleland, *Methods Enzymol.*, 63 (1979) 103–138.
[15] S.H. Eklund, J.F. Robyt, *Carbohydr. Res.*, 177 (1988) 253–258.
[16] D. Su, J.F. Robyt, *Arch. Biochem. Biophys.*, 308 (1994) 471–476.
[17] A. Fersht, *Enzyme Structure and Mechanism*, W.H. Freeman & Co, San Francisco, 1977, p. 95.
[18] K.H. Ebert, G. Schenk, in F.F. Nord (Ed.), *Adv. Enzymol.*, 30 (1968) 179–221.
[19] F. Paul, E. Oriol, D. Auriol, P. Monsan, *Carbohydr. Res.*, 149 (1986) 433–441.
[20] T.P. Binder, G.L. Cote, J.F. Robyt, *Carbohydr. Res.*, 124 (1983) 275–286.