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Interpretation of dextransucrase inhibition at high sucrose concentrations †

Aziz Tanriseven and John F. Robyt *

Department of Biochemistry and Biophysics, Iowa State University Ames, IA 50011 (USA)

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ABSTRACT

When acceptor reactions were carried out at high sucrose concentrations (≥ 200 mM), dextran synthesis was inhibited and the acceptor reactions were increased. A model, based on the known mechanisms of dextran synthesis and acceptor reactions, is proposed to explain the inhibition of dextran synthesis and the increase in the acceptor products at high sucrose concentrations. According to the model, sucrose binds to a third, low-affinity binding site, allosterically changing the conformation of the active site so that dextran cannot be formed but acceptor products can be formed.

INTRODUCTION

The dextransucrase of *Leuconostoc mesenteroides* B-512FM catalyzes the formation of dextran using the glucosyl part of sucrose. B-512F Dextran is a polysaccharide composed of D-glucose units linked by 95% α-(1 → 6) linkages and 5% α-(1 → 3) branch linkages.

It has been shown that dextran synthesis proceeds by a two-site insertion mechanism in which covalent glucosyl and dextranyl ** enzyme intermediates are formed, with dextran polymerization taking place by addition of the glucosyl unit to the reducing end of the growing dextranyl chain[1].

When carbohydrates in addition to sucrose are present in dextransucrase digests, some of the glucosyl groups of sucrose are transferred to the carbohydrates and are diverted from entering dextran[2–5]. These carbohydrates have been called acceptors and include several mono-, oligo-, and poly-saccharides[3,6]. Some accep-

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* Corresponding author.

** Denotes a dextran chain attached through the reducing glucosyl end.

tor products, such as the acceptor products of maltose, isomaltose, and methyl α-D-glucopyranoside, can themselves act as acceptors, and a homologous series of acceptor products are formed[3]. Some acceptors, such as, D-fructose, lactose, and raffinose, give only single acceptor products[3,4].

The formation of acceptor products takes place by the acceptor displacing the glycosyl and/or the dextranyl groups from the covalent enzyme complex[4]. Using B-512FM dextransucrase, we have shown that the acceptors do not compete with sucrose for the sucrose binding sites but bind at a separate acceptor binding site[7]. It has also been shown in our laboratory that the enzyme has two sucrose binding sites and one acceptor binding site[8].

By measuring either the formation of dextran or the release of fructose, it was shown that dextran synthesis follows the Michaelis–Menten equation up to 200 mM sucrose[7,9–13], and thereafter the rate of dextran synthesis decreases. This decrease was interpreted by Ebert and Schenk[13] to be due to the binding of sucrose to an acceptor binding site, where it acts as an acceptor. Stringer and Tsuchiya[10] interpreted sucrose inhibition as a possible competition between sucrose and the acceptor, methyl α-D-glucopyranoside, for a putative acceptor site, since methyl α-D-glucopyranoside relieved the inhibition when the amount of fructose released was measured. Neely[14,15] also explained the inhibition as due to the binding of a second sucrose molecule to the dextransucrase–sucrose complex, in which the enzyme had a greater affinity for the first sucrose molecule than for the second one, with the second sucrose molecule binding to the acceptor site and thus, inhibiting the reaction.

Bovey explained the sucrose inhibition as the binding of a second molecule of sucrose to dextransucrase to form an inactive dextransucrase–glucosyl–sucrose complex[11], although no evidence was presented.

The present work was undertaken to explain sucrose inhibition of dextransucrase because of the various interpretations that have been proposed with essentially no evidence.

EXPERIMENTAL

Chromatography.—Thin-layer chromatography (TLC) was conducted on Whatman K5 plates using three different solvent systems: three ascents of solvent 1, 17:3 (v/v) acetonitrile–water; three ascents of solvent 2, 2:3:5 (v/v/v) nitromethane–water–1-propanol, and two ascents of solvent 3, 17:2:1 (v/v/v) acetone–$CHCl_3$–water. The carbohydrates were visualized by dipping the plates into 5% (v/v) H_2SO_4 in EtOH containing 0.5% thymol and 0.5% α-naphthol, followed by heating for 10 min at 120°C. For the quantitative determination of dextran on the TLC plate by densitometry, the plate was dipped into MeOH containing 20% (v/v) H_2SO_4, followed by heating for 10 min at 120°C.

Enzyme.—Leuconostoc mesenteroides B-512FM dextransucrase was prepared as previously described[16]. The activity of the enzyme was determined by a radiochem-

ical assay[17] using [U-^{14}C]sucrose. Assays were conducted at 25°C and pH 5.4 with 25 mM acetate buffer containing 1 mM $CaCl_2$, 0.1 mg/mL NaN_3, and 0.1 mg/mL Tween 80. The amount of radioactive glucose incorporated into MeOH-insoluble dextran was determined by liquid scintillation spectrometry. The activity is given in international units (IU), namely, the amount of enzyme necessary to incorporate 1 μmol of D-glucose into dextran in 1 min. The activity of the enzyme used was 120 IU/mL and the specific activity was 90 IU/mg of protein.

Enzyme-digest conditions.—The following digests containing 2.4 IU dextransucrase in 25 mM acetate buffer (pH 5.4) were prepared: Digest 1: 5 mL of 50 mM sucrose; Digest 2: 5 mL of 50 mM sucrose containing 50 mM methyl α-D-glucopyranoside; Digest 3: 0.5 mL of 500 mM sucrose; and Digest 4: 0.5 mL of 500 mM sucrose containing 500 mM methyl α-D-glucopyranoside.

The reactions were carried out for 4 h at room temperature (23°C). TLC, using solvent 1, showed that all of the sucrose had been converted into products. At the end of the reaction, Digests 3 and 4 were added to 4.5 mL of acetate buffer so that all of the digests had the same final volume (5 mL) for analysis of the products.

The amount of dextran formed in the digests was determined by separating D-fructose, methyl α-D-glucopyranoside, and acceptor products from the dextran by TLC using three ascents of solvent 2. The carbohydrates were visualized by the H_2SO_4–MeOH method, and the density of the dextran spot at the origin was determined by TLC densitometry using a Uniscan densitometer (Analtech, Inc. Newark, DE). Known amounts (2–10 μg) of dextran T-10 (Sigma, St. Louis, MO) were used as standards. The amounts of methyl α-D-glucopyranoside were similarly determined using two ascents of solvent 3, followed by visualization with the thymol–α-naphthol reagent. Known amounts (1.9–9.7 μg) of methyl α-D-glucopyranoside were used as standards.

RESULTS

Fig. 1 shows the TLC of the reaction digests. Using TLC densitometry, the reaction digests were analyzed as shown in Table I. All of the glucosyl units of sucrose were incorporated into dextran when 50 mM sucrose (Digest 1) was used. In Digest 2 (50 mM sucrose and 50 mM methyl α-D-glucopyranoside), 78% of the glucosyl units of sucrose were incorporated into dextran and 22% of the glucosyl units were transferred to acceptors. When 500 mM sucrose (Digest 3) was used, 94% of the glucosyl units of sucrose were incorporated into dextran and the remaining (6%) were transferrred to fructose, the by-product of the reaction, to form leucrose. In Digest 4 (500 mM sucrose and 500 mM methyl α-D-glucopyranoside), almost all of the glucosyl units of sucrose (95%) were transferred to acceptors, and there was only a small amount (5%) of dextran formed.

To determine the effect of the sucrose concentration on the acceptor reactions, we have used the same amounts of enzyme, sucrose, and methyl α-D-glucopyranoside in Digests 2 and 4 and found that only 8% of the methyl α-D-glucopyranoside

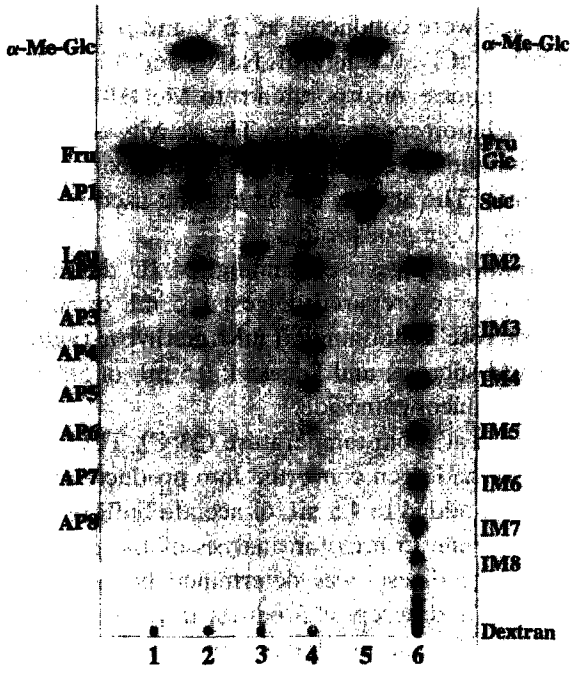

Fig. 1. TLC analysis of dextransucrase digests with different amounts of sucrose and methyl α-D-glucopyranoside. Lane 1: 50 mM sucrose digest; Lane 2: 50 mM sucrose + 50 mM methyl α-D-glucopyranoside digest; Lane 3: 500 mM sucrose digest; Lane 4: 500 mM sucrose and 500 mM methyl α-D-glucopyranoside digest; Lane 5: methyl α-D-glucopyranoside, fructose, glucose, and sucrose standards; Lane 6: isomaltodextrin series standards. [IMn: isomaltodextrins of dp *n*; APn: acceptor product where *n* represents first, second, third, etc., product formed; α-Me-Glc: methyl α-D-glucopyranoside; Glc: glucose; Fru: fructose; Suc: sucrose; Leu: leucrose]. TLC was run on Whatman K5 plates using three ascents of solvent 2 up to the middle of the plate, and then three ascents of solvent 1 to the top of the plate.

TABLE I

Percentages of the total D-glucose from sucrose in products of *Leuconostoc mesenteroides* B-512FM dextransucrase digests

Digests [a]	Dextran (%)	Acceptor product (s) (%)
1.5 mL 50 mM sucrose	100	0
2.5 mL 50 mM sucrose + 50 mM methyl α-D-glucopyranoside	78	22
3. 0.5 mL 500 mM sucrose	94	6
4. 0.5 mL 500 mM sucrose + 500 mM methyl α-D-glucopyranoside	5	95

[a] Each digest contained 2.4 IU of enzyme.

reacted in Digest 2, which consisted of 5 mL of 50 mM sucrose and 50 mM methyl α-D-glucopyranoside, but 39% of the methyl α-D-glucopyranoside reacted in Digest 4, which consisted of 0.5 ml of 500 mM sucrose and 500 mM methyl α-D-glucopyranoside. This result showed that even though the amounts of sucrose, acceptor, and enzyme were the same in the two digests, there was more acceptor products formed in Digest 4 in which the concentration of sucrose was high and the sucrose was inhibiting dextran synthesis.

DISCUSSION

It has been shown by several studies that the rate of dextran formation decreases if the sucrose concentration is greater than 200 mM[7,9–13]. Alsop[18] reported that the size and the amount of dextran synthesized by dextransucrase is dependent primarily on the initial concentration of sucrose. When 2% w/v (58 mM) sucrose was used, almost all of the glucosyl groups of sucrose were incorporated into high-molecular-weight dextran, but when the concentration of sucrose was increased, the amount and molecular weight of dextran decreased and at 400 mM sucrose and above significant amounts of oligosaccharides were formed. The amount of D-glucose, which is obtained when water acts as an acceptor, also increased.

Tsuchiya[19] used 70% (2 M) sucrose and found that the products were mostly oligosaccharides, with some low-molecular-weight dextran ($\leq$ 35 000 Da), and no high-molecular-weigth dextran.

Our results confirmed that the amount of dextran synthesized depends on the sucrose concentration in which concentrations greater than 200 mM give lower amounts of dextran.

We have determined[3,20] that if the concentration of sucrose is kept constant and the ratio of acceptor to sucrose is increased, the amounts of acceptor products are increased and the amount of dextran is decreased. In the present study, we show that when the ratio of acceptor to sucrose is kept constant at 1:1 and the concentration of sucrose is increased, there is a decrease in the amount of dextran formed but there is not a decrease in the amount of acceptor products formed. For example, at a concentration of 50 mM sucrose and 50 mM methyl α-D-glucopyranoside, 78% of dextran was formed along with 22% acceptor products (Table I). When the concentration of sucrose was increased tenfold to 500 mM and the ratio of acceptor to sucrose was kept at 1:1, only small amounts of dextran (5%) were formed, but large amounts of acceptor products (95%) were formed. Similar results have been obtained using maltose as the acceptor[20]. Thus, at high sucrose concentrations, in the presence of equimolar amounts of good acceptors, such as methyl α-D-glucopyranoside or maltose, dextransucrase only catalyzes the synthesis of small amounts of dextran but does catalyze large amounts of acceptor product(s).

Ebert and Schenk[13], Stringer and Tsuchiya[10], and Neely[14,15] each interpreted the sucrose inhibition of dextransucrase by high sucrose concentration (i.e., $\geq$ 200 mM) as the binding of sucrose at the acceptor binding site. Their interpretations

do not explain the increase in acceptor reactions that occur at high sucrose concentrations.

Bovey[11] postulated that dextran synthesis occurs by the formation of a glucosyl–enzyme complex in which the glucose is transferred to a primer. He also postulated that acceptor reactions occur by a different route in which the acceptor reacts with an enzyme–sucrose complex instead of an enzyme–glucosyl complex. Both of these proposed mechanisms have been shown by Robyt et al.[1] and Robyt and Walseth[4] to be incorrect. Based on these incorrect mechanisms, Bovey[11] interpreted inhibition by high sucrose concentrations as the result of sucrose reversibly binding to the enzyme–glucosyl complex, which is the first step in his mechanism of dextran synthesis. This dextransucrase–sucrose–glucosyl complex was postulated to be incapable of further reaction because the sucrose was blocking the active site. Bovey then proposed that the reason acceptor reactions were not affected by high sucrose concentrations was because they were occurring through an enzyme–sucrose complex that could not react with a second sucrose molecule.

In view of what is now known about the mechanisms of dextran synthesis[1] and acceptor reactions[4] of dextransucrase, we propose the following model to explain the simultaneous sucrose inhibition of dextran synthesis and the increased synthesis of acceptor products at high sucrose concentrations. It is known that the active site of dextransucrase has two high affinity sucrose binding sites[1,8] and one acceptor binding site[8]. Acceptors can bind in the acceptor site and react with either the glucosyl or the dextranyl groups that are covalently attached to the X-nucleophilic groups at the enzyme active site[7]. Sucrose does not bind in the acceptor binding site, even at very high sucrose concentrations[7]. At high sucrose concentrations (> 200 mM), it is proposed that sucrose binds at a low affinity, third sucrose binding site, that allosterically changes the conformation of the active site (see B in Fig. 2). This allosteric binding does not affect the binding of sucrose at the sucrose binding sites nor does it affect the formation of glucosyl–enzyme covalent complexes. The binding of sucrose in the allosteric site changes the conformation of the active site in such a way as to prevent the interaction of the two glucosyl groups to give dextran elongation. The allosterically inhibited enzyme, however, still allows an acceptor to bind at the acceptor binding site and interact with one of the glucosyl–enzyme complexes to give acceptor products (see Figs. 2B and C).

Methyl α-D-glucopyranoside and the subsequent acceptor products, which are mainly produced by allosterically inhibited dextransucrase, release glucosyl units from both the uninhibited dextransucrase and the allosterically inhibited dextransucrase. This explains the incorporation of almost all of the glucosyl units of sucrose into acceptor products, rather than into dextran in the digest containing 500 mM sucrose and 500 mM methyl α-D-glucopyranoside.

At high sucrose concentrations, in the absence of acceptors, the allosterically inhibited enzyme can still form dextransucrase–glucosyl complexes, but it does not

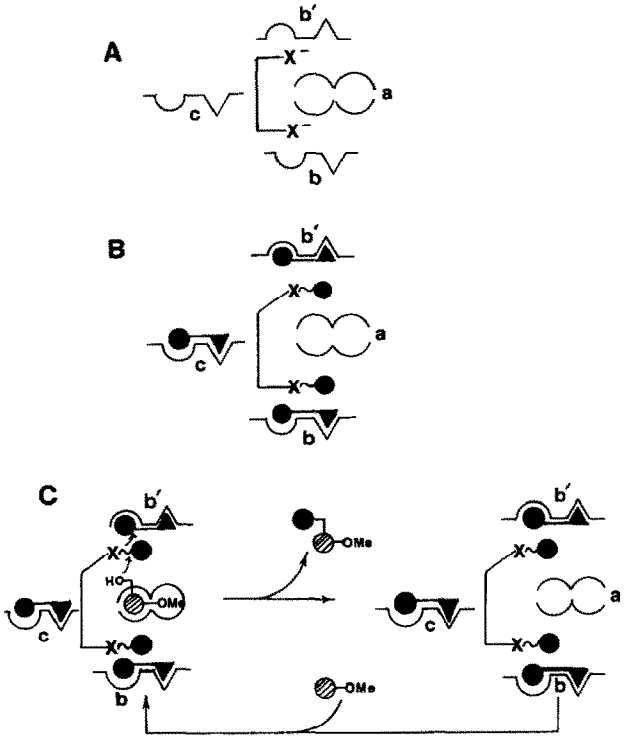

Fig. 2. Model for inhibition of *L. mesenteroides* B-512FM dextransucrase by high sucrose concentrations. (A) Fully active uninhibited dextransucrase active site, showing two high-affinity sucrose binding sites (b and b′) a lower affinity allosteric sucrose binding site (c), and an acceptor binding site (a); (B) allosterically inhibited dextransucrase by high sucrose concentration in which the conformation of the active site has been changed so that dextran synthesis cannot occur; (C) acceptor reaction of allosterically inhibited dextransucrase in the presence of methyl α-D-glucopyranoside acceptor and the formation of acceptor product, methyl α-isomaltoside.

synthesize dextran due to the allosteric change in the conformation of the active site (Fig. 2B). In the absence of acceptors, the glucosyl units can be released from the active site by acceptor reactions with water. This, thus explains the observed[18] increase in D-glucose when the sucrose concentrations are greater than 200 mM. The increase in the amounts of D-glucose, along with the concomitant presence of D-fructopyranose, provide acceptors that also can release D-glucose from the active site and form oligosaccharides that were observed by Alsop[18] and Tsuchiya[19].

In summary, the allosteric sucrose inhibition of dextransucrase explains all of the observed effects of high (> 200 mM) sucrose concentrations: namely, the inhibition of dextran synthesis, the increase in the formation of acceptor products, and the increase in the formation of D-glucose and oligosaccharides.

REFERENCES

1 J.F. Robyt, B.K. Kimble, and T.F. Walseth, *Arch. Biochem. Biophys.*, 165 (1974) 634–640.
2 F.H. Stodola, E.S. Sharpe, and H.J. Koepsell, *J. Am. Chem. Soc.*, 78 (1956) 2514–2518.

3 J.F. Robyt and S.H. Eklund, *Carbohydr. Res.*, 121 (1983) 279–286.
4 J.F. Robyt and T.F. Walseth, *Carbohydr. Res.*, 61 (1978) 433–445.
5 J.F. Robyt and H. Taniguchi, *Arch. Biochem. Biophys.*, 174 (1976) 129–135.
6 J.F. Robyt and S.H. Eklund, *Bioorg. Chem.*, 11 (1982) 115–132.
7 A. Tanriseven and J.F. Robyt, *Carbohydr. Res.*, 225 (1992) 321–329.
8 D. Su and J.F. Robyt, *Carbohydr. Res.*, in preparation.
9 E.J. Hehre, *J. Biol. Chem.*, 163 (1946) 221–233.
10 C.S. Stringer and H.M. Tsuchiya, *J. Am. Chem. Soc.*, 80 (1958) 6620–6625.
11 F.A. Bovey, *J. Polym. Sci.*, 35 (1959) 191–204.
12 J.P. Martinez-Espindola and C.A. Lopez-Munguia, *Biotechnol. Lett.*, 7(7) (1985) 483–486.
13 K.H. Ebert and G. Schenk, *Adv. Enzymol.*, 30 (1968) 179–221.
14 W.B. Neely, *Nature*, 182 (1958) 1007–1008.
15 W.B. Neely and C.F. Thompson, *Nature*, 184 (1959) 64.
16 D. Fu and J.F. Robyt, *Prep. Biochem.*, 20 (1990) 93–106.
17 A.W. Miller, S.H. Eklund, and J.F. Robyt, *Carbohydr. Res.*, 147 (1986) 119–133.
18 R.M. Alsop, *Prog. Ind. Microbiol.*, 18 (1983) 1–44.
19 H.M. Tsuchiya, *Bull. Soc. Chim. Biol.*, 42(12) (1960) 1777–1788.
20 D. Su and J.F. Robyt, Carbohydr. Res., submitted.