Effectors Differently Modulating the Dextransucrase Activity of *Leuconostoc mesenteroides*

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Effects of various compounds on the dextransucrase (EC 2.4.1.5) from *Leuconostoc mesenteroides* was evaluated based on the two catalytic activities of enzyme, that is the hydrolase activity for the substrate, sucrose, and the transferase activity of a D-glucosyl group to an acceptor molecule. The effectors were grouped into six categories by their activation or inhibition of the sucrase and transferase activities of dextransucrase. Type I-A inhibited both activities, type I-B inhibited the sucrase activity, and type I-C inhibited the transferase activity. Type A-A activated both the hydrolase and transferase, and type A-B activated only the transferase. Antagonistic modulation (type IA-A), was shown by methyl α -D-glucoside and glycerol, which activated the sucrase and inhibited the transferase. A double reciprocal plot for dextran gave a biphasic pattern which led to K_i values for each limb. Based on the biphasic kinetics and the action of antagonistic effectors, the regulation of dextran synthesis was discussed.

Dextransucrase (EC 2.4.1.5) catalyzes the transfer of a D-glucosyl group from sucrose to a growing chain of the polysaccharide dextran.¹⁾ In our previous paper,²⁾ we demonstrated that the purified dimer of dextransucrase from *Leuconostoc mesenteroides* NRRL B-1416 was activated by the addition of exogenous dextran (67 μ M), when the enzyme was assayed for the release of reducing sugar (sucrase activity). However, much greater activation was observed when the amount of dextran synthesized (transferase activity) was assayed.³⁾ A discrepancy between the stimulation of two activities should be explained by more extensive studies. Recently, Luzio and Mayer have reported that dextransucrase catalyzed the hydrolysis of the substrate sucrose⁴⁾ and that a glucosylated enzyme had three competing activities, hydrolysis, D-glucosyl-transfer, and polymerization.⁵⁾ In most of the previous papers, the enzyme activity was assayed by measuring either released reducing sugar or synthesized dextran.⁶⁾ To better understand the reaction mechanism, the dextransucrase reaction should be kinetically evaluated for both sucrase and transferase

activities.

In this paper, we describe the effects of various compounds on the dextransucrase activity, which was assayed for both sucrase and transferase activities. Although there have been many reports on various effectors for this enzyme,^{7~10)} there is no systematic characterization of them. Some kinetic analyses with inhibitors were also conducted to have information on the mechanism of enzyme catalysis.

MATERIALS AND METHODS

Materials. Dextransucrase from *Leuconostoc mesenteroides* NRRL B-1416 strain was purified as described previously;³⁾ the enzyme was treated with an endodextranase and the resulting dimeric form was isolated by preparative polyacrylamide gel electrophoresis. A clinical dextran (mol. wt. 75,000 $\pm$ 25,000) was provided by Meito Sangyo Co., Japan.

Enzyme assays. The standard reaction mixture (250 μ l) contained a suitably diluted enzyme (50 μ l), 292 mM sucrose, and 67 μ M clinical dextran in 40 mM acetate buffer (pH 5.2). After incubation at 30°C for 20 min, reactions were stopped by the addition of NaOH (final 0.2 M). Enzyme activity was measured with the following procedures.

Method I. The amount of reducing sugar released from the substrate sucrose was measured by the Nelson-Somogyi method as described previously.¹¹⁾ The sucrase activity was measured by this assay method.

Method II. The amount of D-glucose incorporated into the acceptor dextran was measured as follows: To obtain a sufficient amount of dextran for the subsequent purification step, the reaction was conducted for at least 5 hr. To the reaction mixture (250 μ l) was added two volumes of ethanol (67%, v/v), and polysaccharide was recovered by centrifugation at $1,500 \times g$ for 15 min. The precipitate was dissolved in water (200 μ l) containing 10 mM CaCl_2 , and then three volumes of ethanol (75%, v/v) was added to precipitate the dextran. This precipitation step was repeated thrice. Finally, the precipitate was dissolved in 0.1 M NaOH (240 μ l) and neutralized with 0.1 M HCl (260 μ l). The total sugar (dextran) was measured by the phenol-sulfuric acid procedure as described previously.¹²⁾ This assay corresponded to the transferase activity, and most of the results except for the kinetic experiments were obtained by this procedure. An almost quantitative recovery of the dextran was obtained by this method, which was confirmed by using the clinical dextran as a standard.

Method III. For the kinetic analysis, measurement of synthesized dextran was conducted as follows: The reaction mixture (250 μ l) contained 146 mM sucrose, clinical dextran (up to 13.4 μ M concentration), and enzyme solution. The enzyme reaction was ended by heating for 5 min at 100°C. The resulting mixture was put on a Sephadex G-50 column (0.9 $\times$ 4 cm; in a disposable plastic syringe) and eluted with water. The void volume fraction,

corresponding to 650 to 1150 μ l of eluate, was collected for the assay of dextran by the phenol-sulfuric acid procedure. The recovery of dextran was quantitative in the range of 0 to 1.0 mg of clinical dextran.

RESULTS

Effects of metals and chemicals

Both sucrase and transferase activities were measured in the absence and presence of exogenous dextran (67 μ M clinical dextran), and the latter activity was assayed by the method II described above. The ratio of enzyme activity in the absence and presence of dextran was 1.0 : 4.4 for the sucrase activity, and 1.0 : 8.0 for the transferase activity. The sucrase activity was strongly inhibited by SDS (sodium dodecyl sulfate) (Table I). Although Ca^{2+} slightly activated sucrase activity (Table I), it significantly activated the transferase activity (475 ~ 519%). In contrast, transferase activity was strongly inhibited by EDTA, which had almost no effects on the sucrase activity. A mixture of SDS and Triton X-100 is effective in dissociating dextransucrases from *Streptococcus mutans*.^{13,14)} This mixture activated transferase by 200 ~ 290%, but it caused no sufficient dissociation of *Leuconostoc dex-*

TABLE I. EFFECTS OF METALS AND CHEMICALS ON THE DEXTRANSUCRASE ACTIVITY^a

Compound	Relative activity ^b of			
	Sucrase		Transferase	
	– Dextran	+ Dextran	– Dextran	+ Dextran
None	100	100 ^c	100	100 ^d
CaCl_2 10 mM	100.5	104.6	518.6	475.0
MgCl_2 10 mM	107.9	108.0	—	—
SDS 1 mM	30.9	27.0	—	—
SDS–Triton	145.4	105.5	290.0	200.5
EDTA 10 mM	102.5	99.3	27.1	53.7
2-ME 10 mM	84.5	82.6	—	—
CH_2ICOOH 5 mM	120.0	96.5	109.3	100.0
PCMB 40 μ M	101.3	99.7	—	—

^a Abbreviations used: SDS, sodium dodecyl sulfate; SDS–Triton, a mixture of 0.005% SDS and 0.01% Triton X-100; 2-ME, 2-mercaptoethanol; PCMB, *p*-chloromercuribenzoate.

^b In the presence of clinical dextran (67 μ M).

^c The ratio of sucrase activity obtained in the absence and presence of dextran was 100 : 440, and the latter value was taken as 100 in this table.

^d The ratio of transferase activity obtained in the absence and presence of dextran was 100 : 800, and the latter value was taken as 100 in this table.

TABLE II. EFFECTS OF SUBSTRATE ANALOGS ON THE DEXTRANSUCRASE ACTIVITY

Compound	(mM)	Sucrase		Transferase	
		– Dextran	+ Dextran	– Dextran	+ Dextran
None		100	100 ^a	100	100 ^b
D-Glucose	0.1	41.9	97.2	—	—
	1	20.3	89.0	105.6	93.6
D-Fructose	1	43.2	96.1	102.0	91.5
	50	—	—	74.3	69.1
D-Sorbitol	1	93.4	99.1	99.3	91.1
	50	95.9	116.2	104.3	87.8
Maltose	1	91.9	118.9	87.5	111.7
	50	—	—	25.5	30.9
Maltitol	1	87.7	102.7	90.2	100.0
	50	127.9	117.6	86.3	95.6
D-Glucono- δ -lactone	1	63.5	93.4	60.9	68.1
	50	—	—	36.6	56.4
Methyl α -D-glucoside	0.1	66.2	100.4	—	—
	1	60.8	97.2	96.6	107.4
	50	154.9	114.8	69.4	84.4
	100	208.2	123.5	69.2	78.9
Glycerol	10%	128.1	78.1	47.7	55.7

^{a,b} See the legend for Table I. *c* and *d*, respectively.

transucrases (data not shown). Effects of SH-reagents on this enzyme were rather similar to those described in our previous paper.⁸⁾

Effects of low-molecular-weight sugars

In contrast to the activation of transferase reaction by exogenous dextran (legend for Table I), none of the low-molecular-weight sugars stimulated the transferase activity (Table II). Both D-glucose and D-fructose (1 mM) caused 60~80% inhibition of sucrase activity in the absence of acceptor dextran, which was abolished by the addition of dextran. However, the transferase activity was inhibited to only a small extent by these sugars. The transferase activity was significantly inhibited by maltose (50 mM). Compared with maltitol, sorbitol showed neither activation of sucrase nor inhibition of transferase. Glucono- δ -lactone potently inhibited both activities. For methyl α -D-glucoside and glycerol, the sucrase was stimulated by these sugars at higher concentrations, while addition of acceptor dextran suppressed the activation. Both sugars were rather inhibitory on the transferase.

Kinetics with D-fructose and maltose

Kinetic analysis of D-fructose inhibition was done with varied concentrations of substrate sucrose and dextran. In these experiments, the reaction velocity was expressed as the sucrase activity; the amount of reducing sugar was measured by the method I. Figure 1a shows that D-fructose inhibited the reaction competitively with sucrose and gave a value of *Ki* 44.8 mM. Inhibition of dextransucrase with D-fructose gave a biphasic plot when assayed with various amounts of acceptor dextran (Fig. 1b). In contrast to the competitive inhibition for sucrose, noncompetitive and mixed inhibition were found at higher and lower concentrations of exogenous dextran, respectively. The *Ki* values for two limbs were 283.4 and 107.3 mM, which were more than twice as high as that for sucrose. Similar biphasic kinetics has been observed in measuring the *Km* values for dextran as reported previously.²⁾ Inhibition of dextransucrase with maltose showed the same patterns as those with D-fructose, where *Ki* values were much smaller than those with D-fructose (Table III). Moreover, glucono- δ -lactone was a compe-

FIG. 1. Inhibition of Dextransucrase with D-Fructose.

(a): Double reciprocal plot with different concentrations of substrate sucrose. (b): Double reciprocal plot with different concentrations of substrate dextran. Initial velocity was assayed in the presence (●) and absence (×) of 50 mM D-fructose. The assay was by method I described in the text.

TABLE III. KINETIC CONSTANTS AND MODES OF ACTION OF VARIOUS SUBSTRATE ANALOGS ON THE DEXTRANSUCRASE ACTIVITY

Substrate analog	K_i (mM) for Substrate			
	Sucrose	(Type) ^a	Dextran ^b	(Type) ^a
D-Fructose	44.8	C	107.3	M
			283.4	NC
Maltose	5.4	C	72.0	M
			126.3	NC
D-Glucono- δ -lactone	7.7	C	—	—
Methyl α -D-glucoside	82.4	C	217.4	M
			450.5	NC
Substrate analog	K_a (mM) for Substrate			
	Sucrose	(Type) ^a	Dextran ^b	(Type) ^a
Methyl α -D-glucoside	45.4	M	60.8	M
			187.0	NC

^a C, competitive; M, mixed type; NC, noncompetitive.

^b Double reciprocal plots showed a biphasic pattern.

titive inhibitor of the sucrase activity.

Kinetics with methyl α -D-glucoside

As shown in Table II, methyl α -D-glucoside stimulated the sucrase and inhibited the transferase. Thus, the reaction velocity was measured by respective sucrase and transferase activities. The latter activity was obtained by measuring the amount of dextran synthesized (method III). For sucrase, methyl α -D-glucoside

activated the enzyme in a mixed-type manner giving a K_a value of 45.4 mM for sucrose (Fig. 2a). Although the activation of sucrase was also found for substrate dextran, the biphasic pattern was again observed (Fig. 2b). The K_a values of 187.0 mM (noncompetitive-type) and 60.8 mM (mixed-type) corresponded to the higher and lower concentrations of dextran, respectively (Table III). For the transferase, a competitive inhibition

FIG. 2. Effects of Methyl α -D-Glucoside on the Dextransucrase Reaction.

(a) and (c): Double reciprocal plots with different concentrations of sucrose. (b) and (d): Double reciprocal plots with different concentrations of dextran. Initial velocity was assayed in the presence (●) and absence (×) of 50 mM methyl α -D-glucoside. The assay was by method I for (a) and (b), and method III for (c) and (d).

of enzyme for sucrose was found with a K_i value of 82.4 mM (Fig. 2c). The biphasic inhibition of transferase also gave two inhibition constants for dextran (Fig. 2d). At higher concentrations of dextran, the inhibition was noncompetitive, while mixed inhibition was observed at lower concentrations of dextran.

DISCUSSION

The results shown in Tables I and II indicated that the effectors might be classified into six categories according to the combination of inhibition, activation and lack of effects on the sucrase and transferase activities. Among the inhibitors, glucono- δ -lactone inhibited both sucrase and transferase (type I-A), while D-fructose and EDTA inhibited mainly sucrase (type I-B) and transferase (type I-

C), respectively. As with the *Streptococcus* dextransucrase,¹³ SDS-Triton X-100 activated both activities (type A-A), whereas Ca^{2+} gave strong activation of glucosyltransfer reaction (type A-B). Methyl α -D-glucoside and glycerol showed activation on sucrase and inhibition on transferase (type IA-A). Therefore, each effector has different actions on the dextransucrase reaction, *i.e.*, there are various types of effectors, which specifically modulate the sucrase activity or transferase activity or both. These results clearly support the idea that the dextransucrase reaction should be separated into sucrase and transferase steps with respect to the action of effectors. The ping-pong Bi-Bi mechanism, shown in reaction 1, proposed for the dextransucrase,¹⁵ is not in conflict with the above definition, where the sucrase

activity catalyzes reaction 2. Then, the intermediate D-glucosylated enzyme, which could be recently prepared and characterized by Mayer's group,⁴⁾ reacts with dextran to transfer D-glucosyl residue to the acceptor dextran molecule as shown in reaction 3.

In 1958, Stringer and Tsuchiya reported extensive kinetic studies on the effect of methyl α -D-glucoside on the dextran sucrose reaction.¹⁶⁾ They found that methyl α -D-glucoside increased the $V_{\max}$ value while decreased the yield of high-molecular-weight dextran. More recently, several authors confirmed the dual role of this effector on the dextran sucrose reaction and gave various explanations of the reaction mechanism, mainly based on the efficiency of methyl α -D-glucoside as an acceptor molecule.^{17~21)} However, none of the papers describe the evaluation of this effector for the respective sucrose and transferase activities, which could be distinguishable by the kinetic approach. Our studies on the kinetics of methyl α -D-glucoside indicated that the transferase activity of dextran sucrose was rather inhibited (K_i of 82.4 mM for sucrose), though the sucrose activity was stimulated (K_a of 45.4 mM for sucrose). Among the sucrose analogs tested, D-fructose, maltose, and methyl α -D-glucoside inhibited the transferase, where the double reciprocal plots for varied concentrations of dextran gave sharp downward biphasic patterns (Fig. 1b and 2b, d). The biphasic plots led to K_m (or K_i) values for each limb and the type of inhibition by these analogs was competitive for sucrose, but mixed and noncompetitive for dextran (Table III). These results suggest that dextran sucrose has an allosteric property in the synthesis of dextran molecules. In this respect, it is interesting to note that dextran sucroses from six strains of *L. mesenteroides* had multiple forms of activity,²²⁾ and that enzymes from NRRL B-1299,^{7,8,11)} B-512F,¹²⁾ and B-1416³⁾ were composed of oligomer forms. Since aggregated

oligomer forms of the enzyme showed higher efficiency of glucan synthesizing (transferase) activity,²³⁾ the transferase activity might be regulated allosterically by the inter-conversion of oligomer forms.

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