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NOTE

Acarbose Effect for Dexran Synthesis, Acceptor and Disproportionation Reactions of *Leuconostoc mesenteroides* B-512FMCM Dextransucrase

KIM, DOMAN[1,2]*, KWAN HWA PARK[2], AND JOHN F. ROBYT[3]

[1]*Department of Biochemical Engineering, Chonnam National University, Kwangju, Korea*
[2]*Research Center for New-Biomaterials in Agriculture, Seoul National University, Suwon, Korea*
[3]*Department of Biochemistry and Biophysics, Iowa State University, Ames, IA, U.S.A.*

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Abstract Acarbose effectively inhibited the synthesis of dextran, and the inhibition pattern was a noncompetitive type with a K_i value of 1.35 mM. It also inhibited the disproportionation reaction of dextransucrase with isomaltotriose and decreased the efficiency of the maltose acceptor reaction. Increased concentration of dextransucrase or maltose in reaction digests, however, decreased the degree of inhibition by acarbose.

Key words: Acarbose, dextransucrase, *Leuconostoc mesenteroides*, acceptor reaction, disproportionation reaction

Amino sugar derivatives synthesized by some strains of the *Actinomycetales* sp. inhibit the activity of α-glucosidase [17]. One of these derivatives, acarbose (a pseudotetrasaccharide consisting of an unsaturated cyclitol unit, a 4-amino-4,6-dideoxyglucose unit, and two glucose units), has produced inhibitory effects on glucoamylase [1, 5], α-amylase [2], cyclodextrin glucanosyltransferase (CGTase) [15], maltase [5], and sucrase [14], to mention a few. Acarbose is bound in the active sites of α-amylase and CGTase in such a manner that the α-1,4 glycosidic linkages are not cleaved and transglycosylation cannot occur.

We have investigated the inhibition of acarbose by dextransucrase (EC: 2.4.1.5). The dextransucrases of *Leuconostoc* utilize sucrose as a D-glucosyl donor for the synthesis of dextrans. *L. mesenteroides* NRRL B-512FMCM dextran have main chains that consist of α-(1, 6)-linked D-glucosyl residues [7]. Several sucrose analogs modified at C-6 have been found to inhibit the enzymes of *Leuconostoc mesenteroides* B-512F [6]. Newbrun *et al.* reported that acarbose was a potent inhibitor of GTF from strains of *Streptococcus mutans* and *Streptococcus sanguis* [12]. They reported that the mechanism of action of acarbose was mostly compatible with an interpretation of competitive-type inhibition, although there was significant inconsistency of results for the conclusion. We report herein the inhibition of acarbose in respect to dextran formation, disproportionation- and acceptor-reaction with *Leuconostoc mesenteroides* B-512FMCM dextransucrase which is closely related to *Streptococcus mutans* glucansucrase.

Dextransucrase was produced in a 5-l Fermentor (Bok-Sung Co., Seoul, Korea) in 3 l of LM medium containing 2% (w/v) glucose. The pH and temperature were maintained at 5.5 and 28°C, respectively. The stirring rate was 100 rev/min and there was no aeration. Tween 80, $CaCl_2$, and NaN_3 were added in concentrations of 1 mg/ml, 2 mM, and 0.2 mg/ml, respectively [7]. Dextransucrase was purified as follows. The procedure given here is for a one-liter culture. All procedures were conducted at 4°C. After removing cells by centrifugation, the culture supernatant was concentrated using membrane filtration (30 K cutoff). The concentrated enzyme solution was dialyzed overnight against 8 l of 20 mM sodium acetate buffer (pH 5.2) containing 50 mM NaCl. The dialyzate (about 100 ml) was loaded onto a 1.0×15 cm DEAE-Cellulose column equilibrated with the same buffer. The column was washed with 200 ml of this buffer, and then with 500 ml of 20 mM sodium acetate (pH 5.2) containing 200 mM NaCl. This was followed by 200 ml of 20 mM imidazole-HCl (pH 6.5) containing 200 mM NaCl, and by a linear NaCl gradient (800 ml; 0.2~1.0 M) run over a period of 6 h. The dextransucrase activity was determined by analyzing the rate of fructose released from sucrose (200 mM in 20 mM Na-acetate buffer; pH 5.2; 23°C) [10]. The quantity of fructose on the TLC plates was analyzed with a densitometer as described previously [8, 9, 11].

To test the ability of acarbose to inhibit dextran formation by dextransucrase, a series of reactions were

*Corresponding author
Phone: 82-62-530-1844; Fax: 82-62-530-1849;
E-mail: dmkim@pasteur.chonnam.ac.kr

carried out in 500 µl digests containing sucrose at concentrations between 0.25 mM and 70 mM, acarbose at concentrations between 0.25 mM and 20 mM, and 0.1 U of dextransucrase per ml in 20 mM sodium acetate buffer (pH 5.2) at 28°C. One unit of dextransucrase is defined as the amount of enzyme that catalyzes the formation of 1 µmole of fructose per min at 28°C and pH 5.2. The amount of dextran formed was measured every 5 min for 45 min. The reaction of dextransucrase was terminated by mixing 50 µl of aliquot of the digests with 50 µl of 10% (v/v) pyrridine. The solution was then diluted appropriately with water and 1 µl of the diluted solution was placed on Whatman K6 TLC plates; each TLC plate was irrigated with three ascents of acetonitrile/water, 85/15 (v/v) at room temperature. The plate was thoroughly dried between each ascent. The compounds were developed on the plate by rapidly dipping the plate into a solution containing 3 g of *N*-(1-naphthyl) ethylenediamine and 50 ml of concentrated H_2SO_4 in 1 l of methanol. The plate was dried and then placed in an oven for 10 min at 120°C; blue-black spots appeared on a white background. The maltose acceptor reaction in the presence of different amounts of enzyme was performed in 500 µl digests containing sucrose (50 mM), dextransucrase (1 or 10 U), maltose (15, 30, or 15 mM), and acarbose (0, 5, or 50 mM) in 20 mM sodium acetate buffer (pH 5.2) at 21°C; 25 µl of the digests were mixed with 5 µl of 60 % (v/v) pyridine to stop the reaction. Carbohydrates were analyzed by thin-layer chromatography (TLC) as described above except using a different developing solvent; Nitromethane/1-propanol/water (2:5:1.5, v/v/v). The disproportionation reaction was performed by incubation of B-512FMCM dextransucrase (5 U/ml) and isomaltotriose (100 mM) with or without 10 mM of acarbose in the reaction digest. No sucrose was added. Reaction products were analyzed by TLC as described above.

The cariogenecities of *Streptococcus* sp. in the mouth are related to their abilities to form insoluble glucans by mutansucrase and various glucansucrases. A variety of different compounds have been studied as potential inhibitors of glucansucrases with the hope that an ideal compound could be found that is safe, cheap, and effective in blocking the colonization of *Streptococcus* sp. on teeth. Acarbose interfered with the synthesis of dextran by dextransucrase, a type of glucansucrase. The addition of acarbose inhibited the formation of dextran and it was found that acarbose was a noncompetitive inhibitor, with a K_i of 1.35 mM (Figs. 1a, 1b). The inhibition pattern was different from most other reported compounds such as 6,6'-dithiodisucrose (mixed type) [6] and 6-deoxysucrose, 6-thiosucrose, α-methyl-D-glucoside (competitive type) [4]. Some studies have shown that 3-deoxysucrose, 4-deoxysucrose, and 4-chloro-4-deoxy-galactosucrose are noncompetitive inhibitors for B-512F dextransucrase, but they are very weak inhibitors with K_i values of 530, 201, and 202 mM, respectively [16]. Therefore, acarbose is the strongest noncompetitive inhibitor that has been reported for *Leuconostoc* dextransucrase.

In addition to synthesizing dextran from sucrose, dextransucrase also catalyzes the transfer of glucose from sucrose to other carbohydrates that are presented or are added to the reaction digests [13]. The added carbohydrates are called acceptors and the reaction is called an acceptor reaction. When the acceptor is a monosaccharide or disaccharide, there is usually produced a series of oligosaccharide acceptor products and inhibits the formation of dextran.

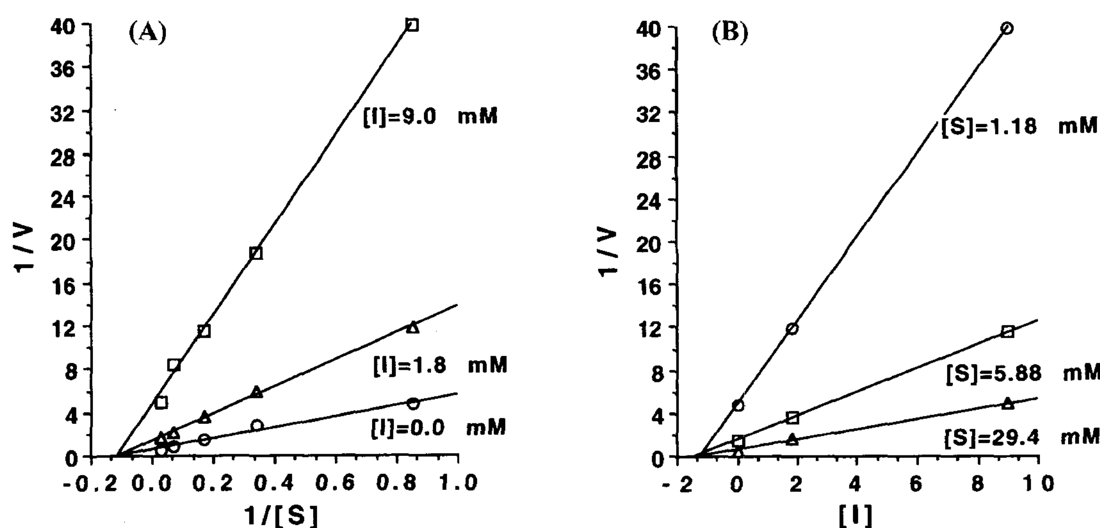

Fig. 1. The Lineweaver-Burk (A) and Dixon (B) plots for the formation of dextran from sucrose by *Leuconostoc mesenteroides* B-512FMCM dextransucrase with or without acarbose in reaction digests.

When maltose and sucrose without acarbose were presented in the dextransucrase reaction digest, panose and isomaltosyl-panose were produced (Fig. 2, lane 8) as acceptor products. If acarbose was added to the reaction digest, no acceptor reaction product was formed (Fig. 2, lane 2). The degree of inhibition of dextransucrase by acarbose was influenced by several factors: First, as the activity of dextransucrase was increased, the amounts and numbers of maltose acceptor products were increased (Fig. 2, compare lane 2 to 3, lane 4 to 5). Second, when the amount of maltose was decreased in the reaction digest, fewer acceptor products formed (Fig. 2, compare lane 2 to 4). Third, also as the amount of maltose was increased, the inhibition by acarbose was overcome (Fig. 2, lane 3 and 5). Fourth, as the amount of acarbose decreased, the number and kinds of acceptor reaction products were increased (Fig. 2, compare lane 2 to 6), and as the dextransucrase activity was increased in the same condition, the inhibition of acarbose was less and a little dextran was formed (Fig. 2, compare lane 6 to 7).

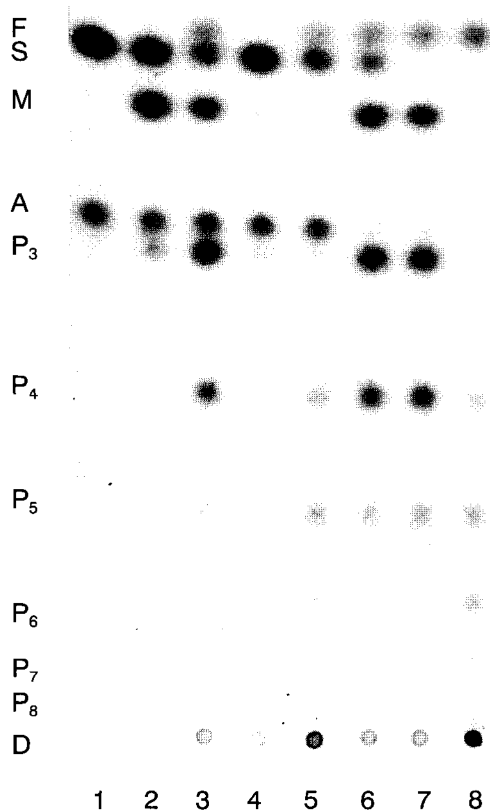

Fig. 2. Acarbose effect for maltose acceptor reaction by *Leuconostoc mesenteroides* B-512FMCM dextransucrase.

Lane 1: Standard for sucrose and acarbose. From lane 2 to lane 7: following values indicate that "U of dextransucrase/concentration of acarbose/concentration of maltose" with 50 mM of sucrose in reaction digest. Lane 2, 1 U/50 mM/150 mM; lane 3, 10 U/50 mM/150 mM; lane 4, 1 U/50 mM/15 mM; lane 5, 10 U/50 mM/15 mM; lane 6, 1 U/5 mM/150 mM; lane 7, 10 U/5 mM/150 mM; lane 8, 1 U/0/30 mM. M, maltose; A, acarbose; S, sucrose; F, fructose; P3, panose; P4, isomaltosylmaltose; P5, isomaltotriosylmaltose; D, dextran; G, glucose.

Reactions in which glucosyl groups are transferred from one saccharide donor to identical, or similar, saccharide acceptors are known as disproportionation reactions (e.g., 2 maltose ⇄ D-glucose+maltotriose), and have been observed for such enzymes as cellulase, amylase, glucoamylase, transglucosylase, glucodextranase, isomaltodextranase, and some glucansucrases [3]. *Leuconostoc mesenteroides* B-512FMCM dextransucrase was found to disproportionate isomalto-oligosaccharides. Figure 3 (lane 1) shows that the products which appear from the reaction with isomaltose are glucose, isomaltose, isomaltotriose, and isomaltotetraose. With isomaltotriose, isomaltotetraose, isomaltopentaose, and isomaltohexaose, further higher-d.p. isomaltooligosaccharides are produced (Fig. 3, lanes 2~5). However, when acarbose was added, the disproportionation reaction was inhibited. (Fig. 3, lanes 1~5).

In summary, we found that the acarbose was an effective noncompetitive inhibitor for *Leuconostoc mesenteroides* B-512FMCM dextransucrase. It also inhibited the acceptor- and disproportionation-reaction as well as dextran formation of the dextransucrase. The degree of inhibition became less as we increased the activity of dextransucrase and the concentration of acceptor in the reaction digest.

Acarbose is an oral oligosaccharide anti-diabetic agent for first-line or adjunctive treatment of noninsulin-dependent diabetes mellitus. It was approved by the US

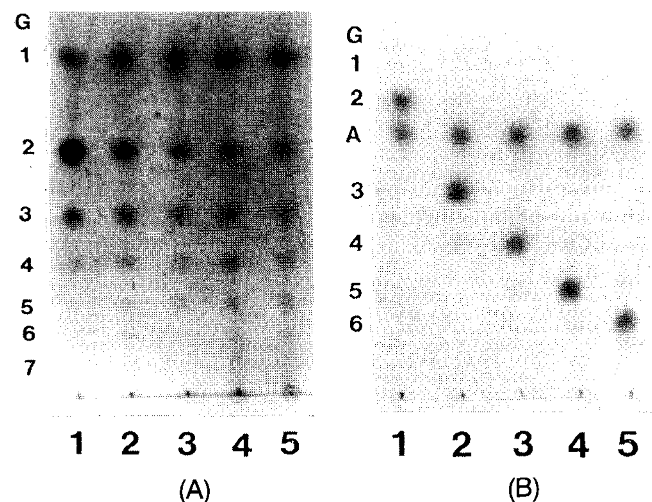

Fig. 3. TLC of disproportionation reaction of isomaltooligosaccharides by *Leuconostoc mesenteroides* B-512FMCM dextransucrase with (A) or without (B) acarbose.

A. Lanes 1 to 5: reaction products from isomaltotriose, isomaltotetraose, isomaltopentaose, isomaltohexaose, and isomaltoheptaose with dextransucrase, respectively. B. Lanes 1 to 5: reaction products from isomaltotriose, isomaltotetraose, isomaltopentaose, isomaltohexaose, and isomaltoheptaose with dextransucrase and acarbose, respectively. G1: glucose, G2 to 7: isomaltose to isomaltoheptaose.

FDA in September 1995 and is available in 35 countries in the world. It could be used as blocking agents for the colonization of cariogenic microorganisms [A1].

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