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Synthesis of acarbose analogues by transglycosylation reactions of *Leuconostoc mesenteroides* B-512FMC and B-742CB dextransucrases

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Abstract

Two new acarbose analogues were synthesized by the reaction of acarbose with sucrose and dextransucrases from *Leuconostoc mesenteroides* B-512FMC and B-742CB. The major products for each reaction were subjected to yeast fermentation, and then separated and purified by Bio-Gel P2 gel permeation chromatography and descending paper chromatography. The structures of the products were determined by one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopy and by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). B-512FMC-dextransucrase produced one major acarbose product, 2^{I}-α-D-glucopyranosylacarbose and B-742CB-dextransucrase produced two major acarbose products, 2^{I}-α-D-glucopyranosylacarbose and 3^{IV}-α-D-glucopyranosylacarbose.

Keywords: Acarbose; Acarbose analogues; Dextransucrase; *L. mesenteroides* B-512FMC; *L. mesenteroides* B-742CB; Transglycosylation reactions

1. Introduction

Dextransucrases [EC 2.4.1.5] elaborated by some *Leuconostoc mesenteroides* strains and *Streptococcus* species catalyze the syntheses of various kinds of dextrans from sucrose.[1] It is also well known that these enzymes catalyze the transfer of glucosyl units from sucrose to other carbohydrates. This reaction is the so-called *acceptor reaction*, and the added carbohydrates are called *acceptors*.[1] More than 30 different carbohydrates are known to act as acceptors, so that it is possible to utilize dextransucrases to synthesize new kinds of carbohydrates by the acceptor reaction.[1,2] Further, different kinds of acceptor products are obtained from the same acceptor by changing glucansucrases elaborated by different species of microorganisms. Dextransucrase from *L. mesenteroides* B-512F produces 6^{III}-*O*-α-D-glucopyranosylmaltotriose and 6^{I}-*O*-α-D-glucopyranosylmaltotriose as the acceptor reaction products from maltotriose.[3] On the other hand, mutansucrase (GTF-I) from *S. mutans* gives 3^{III}-*O*-α-D-glucopyranosylmaltotirose and 3^{I}-*O*-α-D-glucopyranosylmaltotirose in addition to the above two acceptor reaction products from maltotriose produced by *L.* mesenteroides B-512F dextransucrase.[4]

Acarbose is a pseudotetrasaccharide with an unsaturated cyclitol [2,3,4-trihydroxy-5-(hydroxymethyl)-5,6-cyclohexene in a D-*gluco*-configuration] attached to the nitrogen of 4-amino-4, 6-dideoxy-D-glucopyranose, which is linked α-(1→4) to maltose. Acarbose is a strong competitive inhibitor of α-glucosidase,[5–7] alpha-amylase,[5–8] cyclomaltodextrin glucanyltransferase (CGTase),[7,9,10] glucoamylase,[11,12] and glucansucrases.[13,14]

The mechanism of inhibition for these enzymes has been postulated to be due to the cyclohexene ring and the nitrogen linkage that mimics the transition state for the enzymatic cleavage of glycosidic linkages.[6,15]

Some naturally occurring acarbose analogues have been found that have several D-glucose residues attached to the nonreducing-end of acarbose.[5] Yoon and Robyt[16] modified the nonreducing-end of acarbose with specific maltodextrin chains, maltohexaose (G6), maltododecaose (G12), and maltooctadecaose (G18),

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which were added to the C-4-hydroxyl group of the cyclohexene ring by the reaction of acarbose with cyclomaltohexaose in a coupling reaction catalyzed by *Bacillus macerans* CGTase.

The substitution of different saccharides for the maltose unit at the reducing-end of acarbose has given analogues that have significantly increased inhibition and/or altered enzyme specificity.[7,17,18] Park et al.[7,17] reported the formation of several acarbose analogues modified at the reducing-end by the transglycosylation reaction between acarbose and various carbohydrate acceptors catalyzed by *Bacillus stearothermophilus* maltogenic amylase (BSMA). They found that the removal of one D-glucose residue from the reducing-end of acarbose produced acarviosine-glucose, which inhibited yeast α-glucosidase 430-times better than acarbose. They also found that the replacement of the maltose unit by isomaltose gave an inhibitor that inhibited porcine pancreatic alpha-amylase 15 times better than acarbose. Lee et al.[18] found that when the maltose unit of acarbose was replaced by cellobiose or lactose, the acarbose analogues were potent inhibitors for β-glucosidase and β-galactosidase, whereas acarbose was not an inhibitor at all.

In the present study, we report the enzymatic syntheses of new acarbose analogues in which a D-glucopyranosyl residue is added to the 3-hydroxyl group of the cyclohexene ring or to the 2-hydroxyl group of the reducing-end, D-glucose unit by the reaction of acarbose with sucrose in an acceptor reaction catalyzed by dextansucrases from *L. mesenteroides* B-512FMC and B-742CB.

2. Experimental

2.1. Materials

Dextransucrases [EC 2.4.1.5] from *L. mesenteroides* B-512FMC and B-742CB were prepared in our laboratory by previously reported procedures.[19,20] The constitutive mutant (B-742CB/AE4B6) was cultivated statically in a D-glucose medium composed of Bactopeptone (4.4 g/L), yeast extract (4.4 g/L), K_2HPO_4 (20 g/L), glucose (20 g/L), $MgSO_4{\cdot}7H_2O$ (0.17 g/L), NaCl (0.08 g/L), $FeSO_4{\cdot}7H_2O$ (0.08 g/L), $MnSO_4{\cdot}H_2O$ (0.072 g/L), and $CaCl_2{\cdot}2H_2O$ (0.011 g/L) at 21 °C for 24 h. Cells were removed by using a hollow fiber cartridge with a 0.1 μm cutoff (H5MP01-43, Amicon, Inc., Beverly, MA) and the culture supernatant was concentrated and dialyzed against 20 mM pyridinium acetate buffer (pH 5.2) by using a hollow fiber cartridge with a 100 K MW cut-off (Amicon, H5P100-43). The activities of B-512FMC-dextransucrase and B-742CB-dextransucrase were 259 and 13 IU/mL, respectively, using a ^{14}C-sucrose assay.[19] Acarbose was a gift from Dr. K.-H. Park (Dept. of Food Science and Technology, Seoul National University, Suwon, Korea).

2.2. Removal of carbohydrates from reaction mixtures by fermentation, using immobilized yeast

One gram of *Saccharomyces cerevisiae* (commercial Fleishman's bread yeast) was swollen in 5 mL of sterile water for 15 h, and then mixed well with 50 mL of 2.5% (w/v) medium viscosity sodium alginate (Sigma Chemical Co, St. Louis, MO). The mixture was dropped from a tube with a 0.2-0.5 mm orifice into 300 mL of 4% (w/v) $CaCl_2$ solution with stirring and allowed to stand for 3 h at 4 °C to harden the alginate/yeast beads, which were then washed with 300 mL of 20 mM pyridinium acetate buffer (pH 5.2) three times to remove soluble, unwanted material. The immobilized yeast was kept in buffer at 4 °C until use.

2.3. Preparation of dextransucrase acceptor reaction products

Twenty IU of B-512FMC-dextransucrase were added to 2.0 mL of substrate mixture composed of 100 mM acarbose and 100 mM sucrose in 20 mM pyridinium acetate buffer (pH 5.2). The enzyme reaction was carried out at 27 °C for 5 days with the periodic addition of 0.5 mL of 400 mM sucrose solution every 24 h. For B-742CB-dextansucrase, 4 IU of enzyme were added to 4.0 mL of 100 mM acarbose and 100 mM sucrose in 20 mM pyridinium acetate buffer (pH 5.2) buffer, and the reaction was carried out at 37 °C for 3 days. The enzyme reactions were stopped by heating in boiling water for 5 min. The fermentable carbohydrates, mainly unreacted sucrose, D-fructose and D-glucose, in the enzyme reaction digest were removed by yeast (*S. cerevisiae*) fermentation at 37 °C for 24 h, followed by concentration to about 2.0 mL by rotary vacuum evaporation. Any soluble dextran that was in the reaction mixture was removed by adding an equal volume of ethanol (4 °C), followed by centrifugation at 6,000 × g for 10 min and the supernatant was concentrated to about 1.2 mL by rotary vacuum evaporation. The reaction products were analyzed by TLC; an appropriate amount (1-5 μL) of sample was spotted onto a 10 × 20 cm Whatman K5 or K6 silica gel plate (Fisher Scientific, Chicago, IL). The plate was irrigated 2–3 times with 85:20:50:50 (v/v) of acetonitrile–ethyl acetate–1-propanol–water with an 18 cm irrigation path length. The carbohydrates on the TLC plate were visualized by dipping the plate into a MeOH solution containing 0.3% (w/v) *N*-(1-naphthyl)ethylenediamine and 5% (v/v) H_2SO_4, followed by heating at 12 °C for 10 min.[21]

2.4. Fractionation of the dextransucrase reaction products by Bio-Gel P2 column chromatography

About 1.2 mL of the reaction digest was loaded onto Bio-Gel P2 (fine) column (1.5 × 115 cm), and eluted with deionized water at a flow rate of 0.063 mL/min, collecting 1.0 mL fractions. The total carbohydrate content of each fraction was determined by the micro phenol–H_2SO_4 method,[22] and the carbohydrate composition of the fractions was analyzed by TLC as described above with Whatman K6 plates.

2.5. Purification of the dextransucrase reaction products by descending paper chromatography

About 250 μL of the concentrated enzyme reaction products, which were fractionated by BioGel P2 column chromatography, were loaded onto Whatman 3 MM paper (23 × 56 cm).[23] The paper was irrigated with 10:4:3 (v/v) of ethyl acetate–pyridine–water for 36 h to purify B-512FMC-dextransucrase reaction products or 72 h for B-742CB-dextransucrase reaction products.

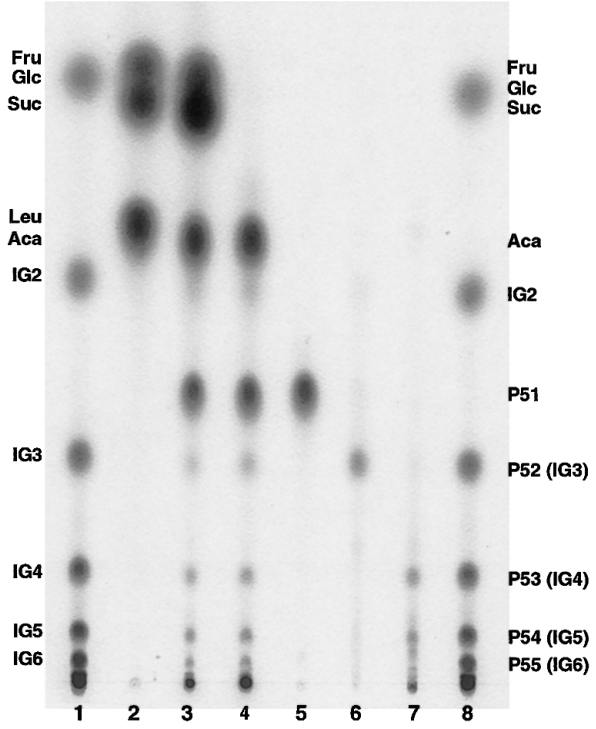

Fig. 1. Thin-layer chromatogram of the dextransucrase (from *L. mesenteroides* B-512FMC) reaction products from sucrose and acarbose. Whatman K5 TLC plates, irrigated three times (path length 18 cm each) with 85:20:50:50 (v/v) MeCN–EtOH–1-propanol–water. Lanes 1 and 8, isomaltodextrin standards; lane 2, carbohydrate standards; lane 3, enzyme reaction products before yeast fermentation; lane 4, enzyme reaction products after yeast fermentation; lane 5, P51; lane 6, P52 (isomaltotriose), lane 7, P53-P55 (isomaltodextrins). Aca designates acarbose and IG_n designates isomaltodextrins having *n* D-glucose residues.

The purified carbohydrates on the paper were located using a $AgNO_3$ reagent, the paper was sectioned, eluted with deionized water, and concentrated to 1 mL by rotary evaporation.[23]

2.6. Analysis of reaction products by matrix-assisted laser desorption ionization-time of flightmass spectrometry (MALDI-TOF MS)[24]

One mg of acarbose and 1.5 mg of the two reaction products (P71 and P72 = P51) were dissolved in 1.0 mL of pure water and filtered through a 0.2 μm membrane; 10 μL of the filtered solution were mixed with 10 μL of 0.1 M 2,5-dihydroxybenzoic acid in MeCN; 1.0 μL of the mixture was transferred to the probe and the solvent was evaporated under *vacuum*. The masses of the compounds in the samples were analyzed by MALDI-TOF MS, using a Dyanmo instrument (Thermo Bio-Analysis, Ltd., Paradise, UK) with a nitrogen laser (337 nm). Ions were detected in a positive mode at an acceleration voltage of 20 kV.

2.7. NMR analysis

About 50 mg of acarbose and about 10–20 mg of the purified enzyme reaction products were exchanged three times with D_2O and were dissolved in 0.5 mL of pure D_2O, and then placed into 5 mm NMR tubes. NMR spectra were obtained on a Bruker DRX 500 spectrometer, operating at 500 MHz for ^1H and at 125 MHz for ^{13}C at 25 °C. Spectra of homonuclear correlation spectroscopy (COSY) and heteronuclear multiple quantum coherence spectroscopy (HMQC) were recorded and analyzed with XWINNMR (Bruker) or NMRVIEW programs.[25]

3. Results

Dextransucrase from *L. mesenteroides* B-512FMC catalyzed an acceptor reaction between acarbose and sucrose, and gave one major product (P51), several minor products (P52–P55), D-fructose, leucrose (5-α-D-glucopyranosyl D-fructopyranose), and isomaltose (Fig. 1, lane 3). When the reaction products were treated with yeast, the fermentable carbohydrates, D-fructose and sucrose, were removed (Fig. 1, lane 4). The remaining reaction products were fractionated by Bio-Gel P2 gel permeation chromatography (Fig. 2). Unreacted acarbose and minor reaction products (P52–P55) were well separated, but the major reaction product, P51, was co-eluted with isomaltose (IG2) and leucrose (Leu). A51 was separated from IG2 and Leu by descending paper chromatography (Fig. 1, lane 5). The minor reaction products (P52–P55) were determined to be isomaltodextrins by TLC R_G values (Fig. 1, lanes 6 and 7).

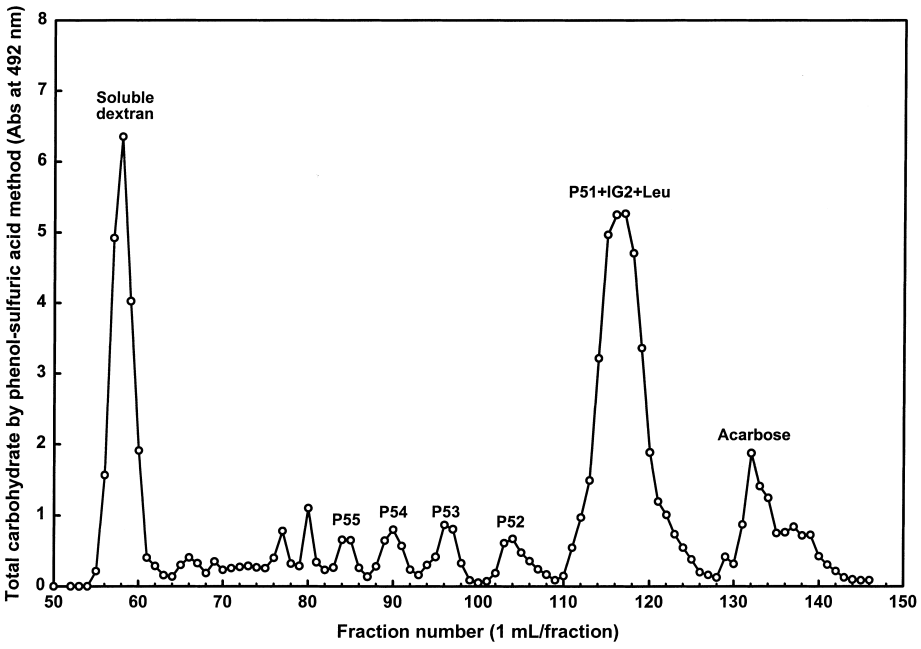

Fig. 2. Bio-Gel P2 gel permeation column chromatogram (1.5 × 115 cm; flow rate 0.063 mL/min; fraction size, 1.0 mL) of the *L. mesenteroides* B-512FMC dextransucrase reaction products.

Dextransucrase from *L. mesenteroides* B-742CB gave two major acceptor reaction products (P71 and P72), along with other minor products, and D-fructose and D-glucose (Fig. 3, lane 3), which were removed by yeast fermentation (Fig. 3, lane 4). The products remaining after yeast fermentation were fractionated by Bio-Gel P2 gel permeation chromatography. Acarbose was separated from the two major reaction products, P71, P72, and leucrose, which eluted together. P71, P72, and leucrose were separated and purified by a descending paper chromatography (Fig. 3, lanes 5 and 6).

The number of D-glucose units that were added to acarbose for products P71 and P72 were determined by using MALDI-TOF MS. The results are shown in Fig. 4 in which the masses of P71 and P72 have been increased over that of acarbose by exactly a single D-glucose residue.

Acarbose and the acceptor reaction products, P51, P71, and P72, were analyzed by ^{1}H, ^{13}C, ^{1}H-COSY and $^{1}H/^{13}C$-HMQC NMR.[16] The analysis of the anomeric proton (H-1) of P71 displayed a new anomeric proton (V-1 in Fig. 5B) at 5.27 ppm, which was very close to the III-1 proton (5.24 ppm) of acarbose (Fig. 5A). The Roman numerals refer to the particular carbohydrate residue, starting with the reducing-end and the Arabic numerals refer to the particular carbon on the residue, as shown in Fig. 7. When the integral of IV-6 proton was made 1, the total integrals of III-1 proton and V-1 proton was 2. The ^{13}C chemical shift of the new anomeric carbon (C-1) appeared at 99.2 ppm (data not shown). These results indicate that one molecule of D-glucose was added to acarbose with an α-linkage.[26,27] The $^{1}H/^{13}C$-HMQC NMR spectrum of P71 (Fig. 7B) was almost identical to that of acarbose (Fig. 7A) with the exception of the 5-hydroxymethyl cyclohexene ring (unit IV in Fig. 7A and B) and the D-glucopyranosyl unit (unit V in Fig. 7B). The changes of the carbon-chemical shifts of the 5-hydroxymethyl-cyclohexene ring before and after the addition of D-glucopyranose to acarbose were, respectively, as follows; C-1, 56.5–56.2; C-2, 71.5–69.0; C-3, 73.3–79.5; C-4, 71.1–70.1; C-5, 139.4–139.0; C-6, 124.0–124.6; C-7, 62.0–62.0 ppm (Fig. 6A and B) with a significant downfield chemical shift of 6.2 ppm for C-3 and a relatively small upfield chemical shift at C-2 of 2.5 ppm, and 1.1 ppm for C-4 of the 5-hydroxymethyl cyclohexene ring. These chemical shifts are characteristic of the attachment of a D-glucopyranosyl unit to the C-3 position of 5-hydroxylmethyl cyclohexene ring (unit IV in Fig. 7B) of acarbose.[16] Thus, the NMR results indicate that the D-glucopyranose unit was attached to the 5-hydroxymethyl cyclohexene ring by an α-(1 → 3) linkage.

P72 and P51 were the same compounds, because they both gave the same ^{1}H and ^{13}C NMR spectra. When the anomeric proton of P51 was analyzed, two new anomeric proton peaks appeared at 5.03 and 5.33 ppm chemical shifts, V(I(α))-1 and V(I(β))-1 in Fig. 5(C). This indicates that a new anomeric proton is present on acarbose, which has an α-configuration of the D-glucose residue that is attached to the reducing-end D-glucose unit (I in Fig. 7C), as compared with unsubstituted acarbose (Fig. 5A). When the integral of the IV-6 proton (the chemical structures refer to those in Fig. 7) was 1, the integrals of the new anomeric proton peaks

were 0.5 for each proton. This indicates that one D-glucopyranosyl unit was attached to acarbose, but that there are two forms of the newly bound D-glucopyranosyl unit due to the α- and β-anomeric structures at the reducing-end. The ^{13}C chemical shifts of the new anomeric carbons, V(I(α))-1 and V(I(β))-1, appeared at 96.6 and 98.1 ppm (data not shown).

The $^{1}H/^{13}C$-HMQC NMR spectrum of P51 (Fig. 6C) was almost identical to that of acarbose (Fig. 6A) with the exception of the reducing-end D-glucose (unit I in Fig. 7A and C) and the D-glucose unit (unit **V** in Fig. 7C). The changes of the carbon-chemical shifts of the reducing-end α-glucose residue (unit I) before and after the addition of D-glucopyranose to acarbose were respectively as follows; C-1, 92.3–89.6; C-2, 71.9–76.0; C-3, 73.7–71.8; C-4, 77.5–76.5; C-5, 70.3–70.1; C-6, 61.0–61.0 ppm (Fig. 5A and C). And those of the reducing-end β-glucose residue (unit I) before and after the addition of D-glucopyranose were, respectively, as follows; C-1, 96.1–96.5; C-2, 74.3–78.8; C-3, 76.6–75.3; C-4, 77.5–76.5; C-5, 75.0–74.8; C-6, 61.0–61.0 ppm (Fig. 6A and C). There were relatively large downfield chemical shifts of 4.1 ppm for C(α)-2 and 4.5 ppm for C(β)-2, respectively, and relatively small changes for the chemical shifts at C(α and β)-1 and at C(α and β)-3 of the reducing-end glucose unit. These chemical shifts are characteristic of the attachment of a D-glucopyranosyl unit to the C-2 position of the reducing-end glucose residue (unit **I** in Fig. 7C) of acarbose. These results show the formation of two forms of anomeric carbons and anomeric protons when the D-glucopyranosyl unit is attached to I-2 and would be found for α-and β-kojibiose.[28] The NMR results, thus, indicate that the D-glucopyranose is attached to the reducing-end glucose unit of acarbose by an α-(1→2) linkage (Fig. 7C).

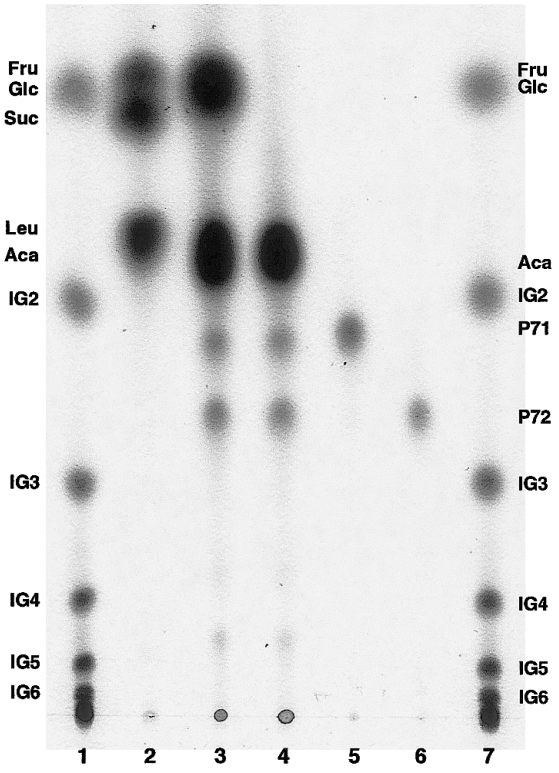

Fig. 3. Thin-layer chromatogram of the *L. mesenteroides* B-742CB dextransucrase reaction products from sucrose and acarbose. Whatman K5 TLC plates, irrigated three times (path length 18 cm each) with 85:20:50:50 (v/v) MeCN–EtOH–1-propanol–water. Lanes 1 and 7, isomaltodextrin standards; lane 2, carbohydrate standards; lane 3, enzyme reaction products before yeast fermentation; lane 4, enzyme reaction products after yeast fermentation; lane 5, P71; lane 6, P72. Aca designates acarbose and IG_n designates isomaltodextrins having *n* D-glucose residues.

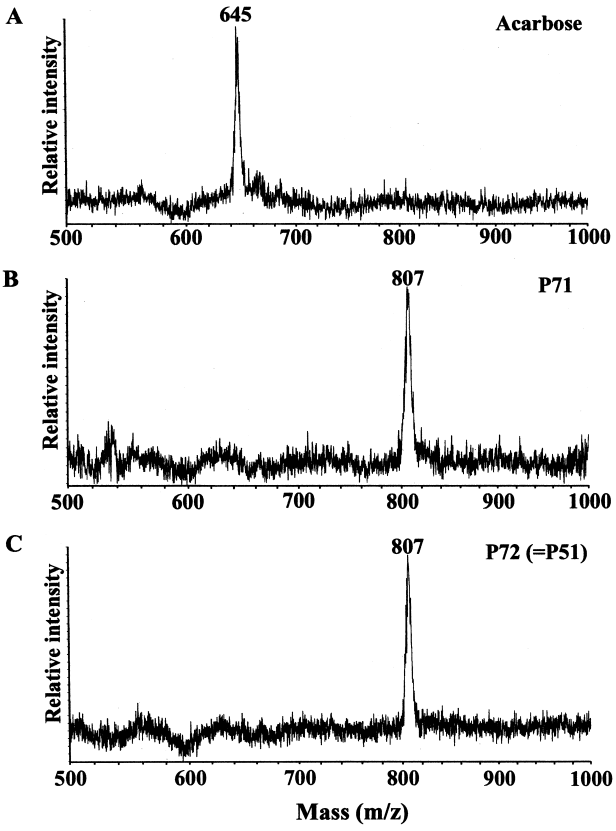

Fig. 4. Matrix-assisted laser desorption ionization-time of flight mass spectrometric (*MALDI-TOF MS*) analysis of the number of D-glucose units added to acarbose by the dextransucrase transglycosylation reactions. A, spectrum of acarbose; B, spectrum of P71 product; C, spectrum of P72/P51 products.

4. Discussion

B-512FMC-dextransucrase produces a dextran composed of 95% of α-(1→6) linkages (main chains) and 5% α-(1→3) branch linkages from sucrose.[1] When acceptor molecules are present in the reaction mixture,

B-512FMC-dextransucrase primarily transfers the D-glucose residue from sucrose to the 6-hydroxyl group of monosaccharides and to the nonreducing-end 6-hydroxyl group of di- and higher-saccharides.[1,29] The first acceptor product of D-glucose is isomaltose. Isomaltose can also act as an acceptor to give isomaltotriose, and isomaltotriose can also act as an acceptor to give isomaltotetraose and eventually a series of isomaltodextrins are formed.[1,29] When maltose is the acceptor, the trisaccharide, panose (6^{II}-α-D-glucopyranosylmaltose) is the product; this trisaccharide is also an acceptor and eventually a series is produced with chains of isomaltodextrins attached to maltose at the 6-OH group of the nonreducing-end D-glucose.

Fu and Robyt[3] found that B-512F dextransucrase transferred D-glucose from sucrose to the 6-OH groups of both the nonreducing-end and the reducing-end D-glucose moieties when maltotriose to maltooctaose were the acceptors. The nonreducing-end products predominated and they were the only ones that were further elongated, as the reducing-end product contained only a single D-glucose residue. B-512F dextransucrase gave

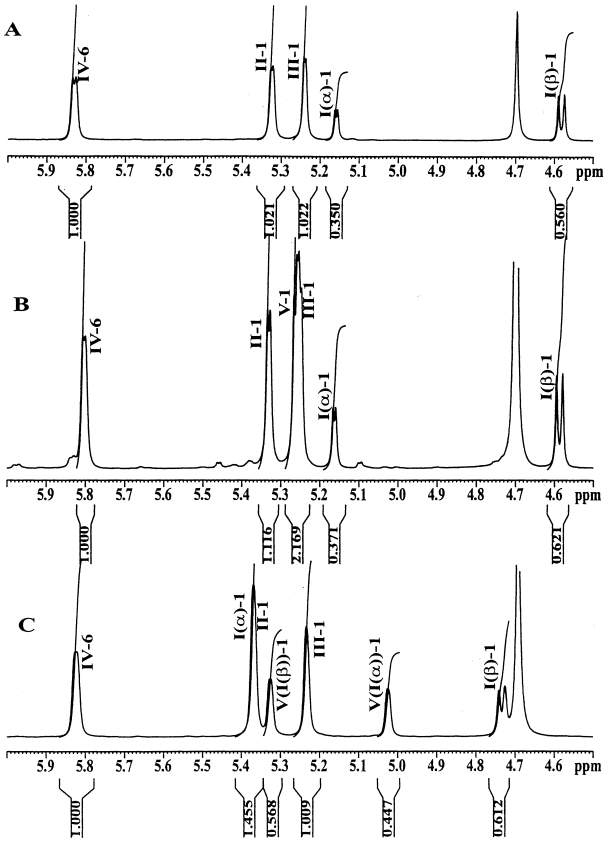

Fig. 5. ^{1}H NMR spectra of anomeric proton regions. A, acarbose; B, P71; C, P72 and P51. The NMR peaks are designated first by the Roman numeral of the residue, starting with the reducing residue at **I**, and followed by the particular proton position on the residue. For example, I(α)-1 indicates that the anomeric proton of unit I has the α-configuration.

unusual acceptor products when cellobiose, lactose, and raffinose were the acceptors.[1,29] The enzyme exclusively transferred D-glucose from sucrose to the C-2-hydroxyl group of the reducing-end D-glucose moiety of cellobiose and then transferred D-glucose to the 6-OH group of the first transferred D-glucose moiety of cellobiose to eventually give a series of isomaltodextrins attached α-(1→2) to the reducing-end moiety.[1,29] When lactose was the acceptor, the enzyme also transferred D-glucose from sucrose to the 2-OH of the D-glucose moiety at the reducing-end. This D-glucose unit, however, was not elongated as it was for cellobiose. When raffinose was the acceptor, the enzyme transferred D-glucose from sucrose to the 2-OH group of the D-glucose residue and did not elongate it. It, thus, appears that the presence of a β-glycosidic bond in the acceptor changed the specificity of B-512F dextransucrase to give transfer to the 2-OH group at the reducing-end glucose rather than transfer to 6-OH at the nonreducing-end and when D-galactose was present, as it is for lactose and raffinose, only a single D-glucose unit is transferred. B-512F dextransucrase and B-512FMC dextransucrase, the enzyme from the constituent, high-producing mutant, give identical dextrans and acceptor products.

In present study, we have found that B-512FMC-dextransucrase also transferred D-glucose from sucrose to the 2-OH of the reducing-end moiety of acarbose to give 2^{I}-*O*-α-D-glucopyranosylacarbose (P51 and P72 in Fig. 7C) as an acceptor reaction product. This was not the expected product as acarbose does not have either a β-glycosidic bond or a D-galactose moiety in its structure. Acarbose contains a pseudocarbohydrate, [2,3,4-trihydroxy-5-(hydroxymethyl)-5,6-cyclohexene in a D-gluco-configuration] that is attached to the nitrogen of 4-amino-4,6-dideoxy-D-glucose. It is possible that these unusual structures are not bound in the enzyme acceptor-binding site and that only the maltose moiety of acarbose at the reducing-end is bound, thus giving transfer to the 2-OH group of the reducing-end D-glucose moiety of the maltose unit. Acarbose is also known as an inhibitor of B-512FMC dextransucrase[13] and this could be a determining factor on the type of acceptor product that is formed.

B-742CB-dextransucrase catalyzes the synthesis of a highly branched dextran that is composed of α-(1→6) linked D-glucose in the main chains with a major amount of α-(1→2) linked single branch D-glucose residues and smaller amounts of α-(1→3), long-chain branch linkages.[19,30] In this study, B-742CB-dextransucrase produced two acceptor reaction products with acarbose, 3^{IV}-*O*-α-D-glucopyranosyl acarbose (P71 in Fig. 7B) and 2^{I}-*O*-α-D-glucopyranosyl acarbose (P72 in Fig. 7C). The second has the same structure as the acceptor product (P51) produced by B-512FMC-dextransucrase. The first product (P71) had D-glucose at-

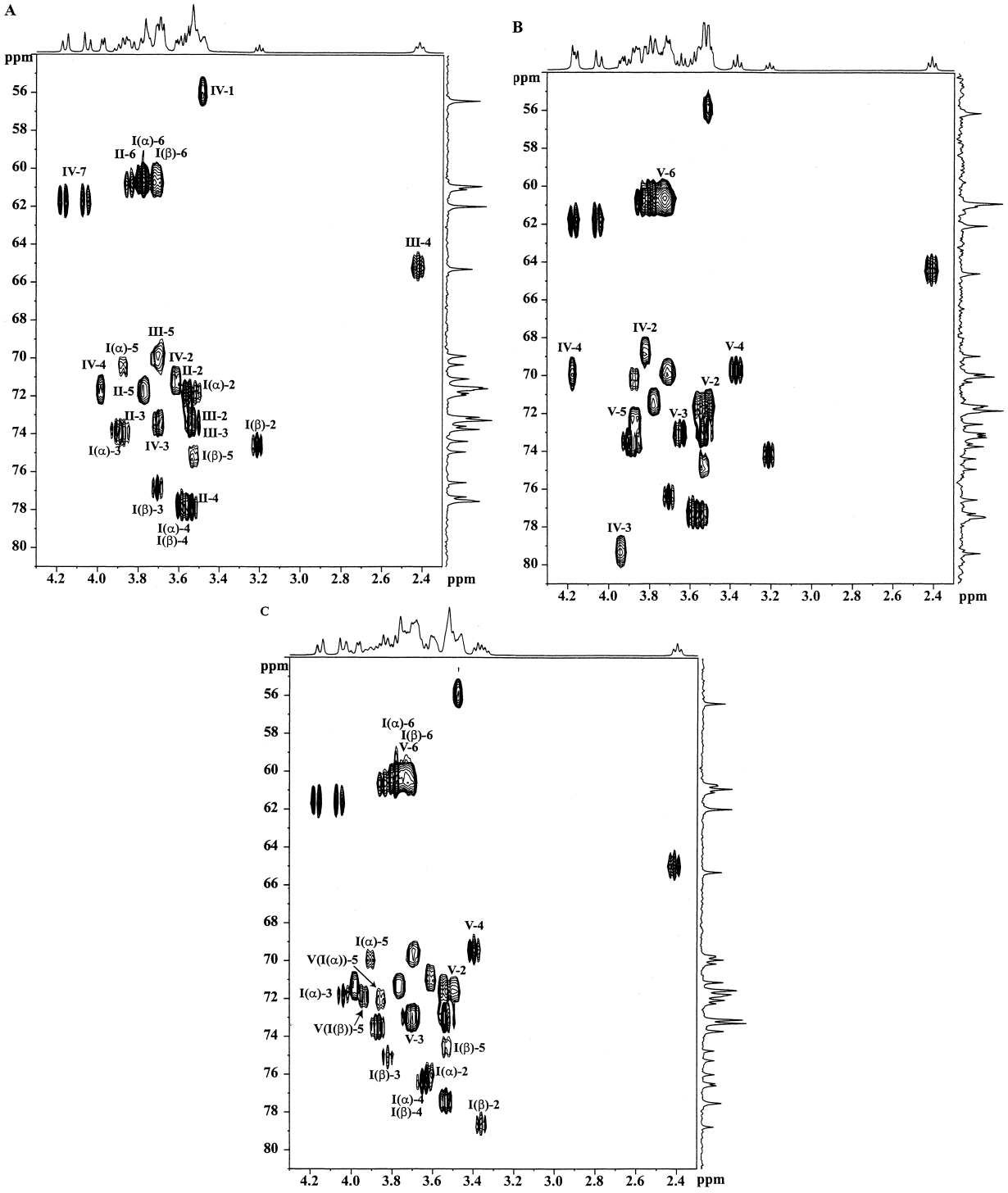

Fig. 6. HMQC NMR spectrum of acarbose (A), P71 (B) and P72 and P51, (C) recorded in D_2O at 25 °C. The NMR peaks are designated first by the Roman numeral of the residue, starting with the reducing residue at **I**, followed by the particular carbon/proton position on the residue. Chemical structures of acarbose and its analogues refer to Fig. 7A, B, and C, respectively.

tached α-(1→3) to the 3-hydroxyl group of the nonreducing-end, cyclohexene ring. Acceptor products of B-742CB have not been studied extensively, so it is not possible to say whether or not the formation of the α-(1→3) linkage at the nonreducing-end of acarbose is unusual. The presence of the pseudo-D-glucose structure without the ring oxygen and with the C-5,6 unsaturated linkage at the nonreducing-end might be a determining factor in the unusually linked acceptor product.

Mutansucrase (glucosyltransferase-insoluble, GTF-I) from *S. mutans* 6715 synthesizes an α-(1→3) linked

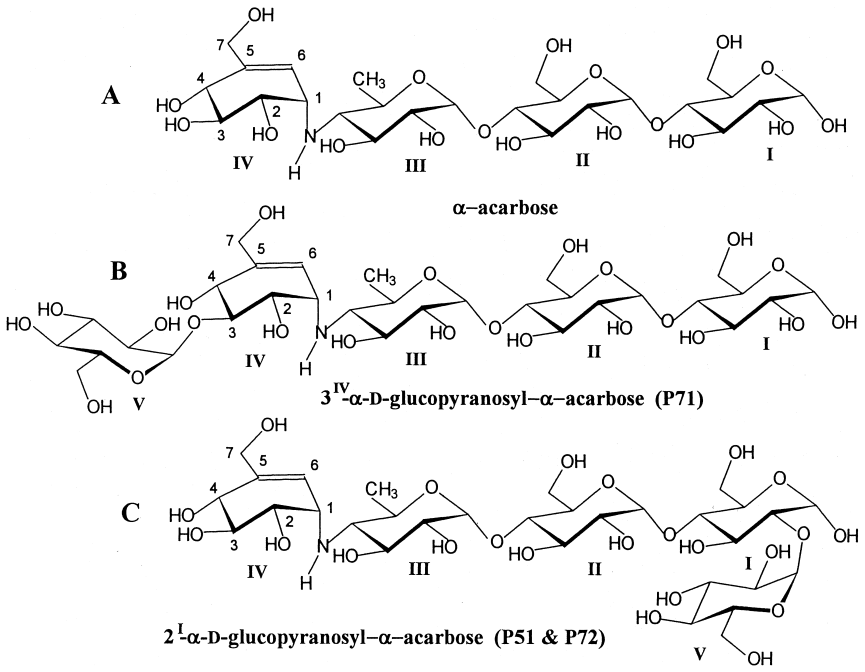

Fig. 7. Proposed chemical structures of (A) acarbose, (B) P71, and (C) P51 and P72. Each of the residues of acarbose is designated by Roman numerals, starting with I at the reducing-end residue.

glucan that is not branched.[1] It also produces four kinds of acceptor reaction products from maltotriose, transferring D-glucose from sucrose to the reducing-end or to the nonreducing-end, forming α-(1→3) and α-(1→6) linkages at both ends:[1,3] 6^{III}-*O*-α-D-glucopyranosyl maltotriose, 6^{I}-*O*-α-D-glucopyranosylmaltotriose, 3^{III}-*O*-α-D-glucopyranosyl maltotriose and 3^{I}-*O*-α-D-glucopyranosylmaltotriose.[4] The former two acceptor reaction products are also made by B-512F-dextransucrase, but the latter two are only produced by mutansucrase. Besides mutansucrase, alternansucrase is the only other glucansucrases that is known to form α-(1→3) linkages at the nonreducing-end of an acceptor D-glucose residue. But for alternansucrase, this D-glucose residue must be linked α-(1→6) to another D-glucose residue. For example, alternansucrase only forms panose (6^{II}-α-D-glucopyranosylmaltose) from maltose but forms an α-(1→3) linkage to isomaltose to give 3^{II}-α-D-glucopyranosylisomaltose.[31]

In conclusion, we have used two dextransucrases from *L. mesenteroides* B-512FMC and B-742CB that have different transglycosylation specificities to synthesize two acarbose analogues. Both enzymes transferred D-glucose from sucrose to the 2-OH group of the reducing-end moiety of acarbose to give an analogue with D-glucose linked α-(1→2). In addition, B-742CB dextransucrase also transferred D-glucose to the C-3-OH group of the nonreducing-end moiety of acarbose to give an analogue with D-glucose linked α-(1→3).

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