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Dextran acceptor reaction of *Streptococcus sobrinus* glucosyltransferase GTF-I as revealed by using uniformly ^{13}C-labeled sucrose

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Abstract

A sucrose glucosyltransferase GTF-I from cariogenic *Streptococcus sobrinus* transferred the uniformly ^{13}C-labeled glucosyl residue ([U-^{13}C]Glc) from [U-^{13}C]sucrose to exogenous dextran T500 at the nonreducing-end, mostly by α-(1→6) linkages and partially by α-(1→3) linkages, as revealed by the ^{13}C–^{13}C NMR coupling pattern. With increasing amounts of [U-^{13}C]sucrose, transfer of [U-^{13}C]Glc to the α-(1→3)-linked chain became predominant without increase in the number of chains. The transfer of [U-^{13}C]Glc to an isomaltopentaose acceptor occurred similarly to its transfer to T500. α-(1→3)-branches in the [U-^{13}C]dextran, specifically synthesized from [U-^{13}C]sucrose by a *Streptococcus bovis* dextransucrase, were not formed by GTF-I, as judged by the observation that a newly-formed α-1,3,6-banched [U-^{13}C]Glc was not detected, which could have been formed by transferring the unlabeled Glc from sucrose to the internal α-(1→6)-linked [U-^{13}C]Glc at C-3. The ^{13}C–^{13}C one-bond coupling constants (^{1}J) were also recorded for the C-1–C-6 bond of the internal α-(1→6)-linked [U-^{13}C]Glc and of the nonreducing-end [U-^{13}C]Glc.

Keywords: Dextran; Dextransucrase; Mutan; Mutansucrase; Sucrose glucosyltransferase; Glucosyltransferase; *Streptococcus sobrinus*; D-[U-^{13}C]sucrose; ^{13}C–^{13}C coupling

1. Introduction

Cariogenic *Streptococcus sobrinus* secretes three dextransucrases (GTF-S, EC 2.4.1.5)[1] and one (1→3)-α-D-glucan-synthesizing enzyme (sucrose glucosyltransferase GTF-I, mutansucrase GTF-I, EC 2.4.1.),[2] which cooperatively synthesize adherent water-insoluble glucan from sucrose.[3] The activity of a purified GTF-I is extremely low,[4] but is highly stimulated with exogenous dextran as well as with the endogenous dextran produced by GTF-S.[5,6] The GTF-I was suggested to form branch points by transferring glucosyl residues (Glc) from sucrose to the internal α-(1→6)-linked Glc of the dextran at the 3-hydroxyl group.[4] The GTF-I then would elongate α-(1→3)-linked side chains in the dextran by transferring Glc to the branch points. The stimulating effect of dextran could be attributed to the growing α-(1→3)-linked side chains of higher nigerooligosaccharides.[7,8] However, the average number of Glc residues between the branch points was not decreased by the GTF-I action, suggesting no formation of branches by this enzyme.[9] The branched glucan was also suggested to be formed by transferring the α-(1→3)-glucan chain directly to various positions along the dextran chain.[10]

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In this study, we used [U-^{13}C]sucrose in order to distinguish the transferred Glc from the acceptor Glc, as used in our previous report,[8] and found that most of the [U-^{13}C]Glc is transferred to the 6-hydroxyl group at the nonreducing-end Glc until the α-(1→3)-linked [U-^{13}C]Glc side chains higher than approximately a pentamer were formed in the dextran. A specifically synthesized [U-^{13}C]dextran acceptor was also used to detect a shift of the internal α-(1→6)-linked [U-^{13}C]Glc to the α-1,3,6-branched [U-^{13}C]Glc by transfer of the Glc from unlabeled sucrose to the 3-hydroxyl group.

2. Experimental

Carbohydrates and reagents.—[U-^{13}C]Sucrose (min 99%) was purchased from ISOTEC Inc. (Miamisburg, OH), dextran T10–T2000 from Pharmacia, and isomaltooligosaccharides of dp 2–7 from Seikagaku Co. (Tokyo, Japan). Leucrose was a gift from Hokuren Federation of Agricultural Cooperatives (Japan). [U-^{13}C]Dextran (11.2 mg) was de novo synthesized from [U-^{13}C]sucrose (100 mg) for 23 h at 37 °C by a purified *S. bovis* 9809 dextransucrase (30 μg) in 1 mL of 50 mM sodium phosphate buffer (pH 6.8) containing 0.01% of NaN_3 and 0.1% of Triton X-100. The dextransucrase (30 U/mg protein) from the culture supernatant was obtained by chromatography on Bio-Gel hydroxyapatite and DEAE-Toyopearl as reported,[11] and was the intact form with M_r 16.5 kDa and pI 3.7. During the steps for preparation of [U-^{13}C]dextran, care was taken to remove by centrifugation a water-insoluble sediment from the [U-^{13}C]sucrose preparation and also to thoroughly remove mono- and oligosaccharides from the [U-^{13}C]dextran preparation by five washes with 4.5 mL of 75% EtOH. The M_r value of the [U-^{13}C]dextran was estimated by gel filtration on Bio-Gel A-50m in 0.5 M NaOH at near 0 °C, using T150–T2000 as M_r standards.

GTF-I enzyme and enzyme assay.—The GTF-I from the culture supernatant of *S. sobrinus* strain 6715 was purified by chromatography on Bio-Gel HP (Bio-Rad Laboratories) and DEAE-Toyopearl (Tosoh Corporation, Japan), followed by preparative isoelectric focusing using a LKB8100 Ampholine electrofocusing column, as already reported.[8] The purified GTF-I preparation (10.5 U/mg protein) was the intact form of GTF-I with M_r 16.5 kDa and pI 4.9, to which was added PMSF (0.1%), merthiolate (0.1%), and EDTA (0.1%), and it was kept near 0 °C until use.

All reactions were done at 37 °C in 50 mM sodium phosphate buffer, pH 6.8, containing 0.01% of NaN_3 and 0.05% of Triton X-100.[8] The insoluble glucan formed was measured by the phenol–H_2SO_4 method.[12] One unit (U) of insoluble-glucan-synthesizing activity was the amount of enzyme catalyzing the incorporation of 1 μmol of glucose from sucrose into insoluble glucan per min. Protein was determined by the Lowry method.

TLC analysis.—Acceptor products were formed for 1 h in 20 μL of the buffer to which was added 1 μM GTF-I, 100 mM sucrose, and 100 mM (Glc equiv) acceptor, and they were separated by thin-layer chromatography (TLC) with three ascents on Silica Gel 70 (Wako Pure Chem., Japan) in 15:3:4 (v/v/v) 1-butanol–pyridine–water at rt. The solvent path-length was 18 cm, and the time of each ascent was ~8 h. The sugars were stained by spraying the plates with 80% (v/v) H_2SO_4, followed by heating for 5 min at 120 °C, as previously reported.[8]

^{13}C NMR analysis.—Dextran acceptor products were formed for 1 h in 1 mL of the buffer containing 1 μM GTF-I, 1–10 mM [U-^{13}C]sucrose and 100 mM (Glc equiv) dextran T500 (16.2 mg). The products were collected by centrifugation and washed four times with 6 mL of 67% EtOH solution containing 33 mM NaCl. The addition of salt was necessary, since the dextran was partially soluble in EtOH solutions of low ionic strength.[13] The total yields after lyophilization were 12.6–14.1 mg. The samples were suspended in 0.4 mL of water, or dissolved in 0.4 mL of 0.5 M NaOH, containing 10% (v/v) D_2O and 1% sodium 4,4-dimethyl-4-silapentane-1-sulfonate (E. Merck). The ^{13}C NMR spectra were recorded with 77,000–166,000 scans at 67.9 MHz on a JNM-GX270 spectrometer (Jeol,

Japan) and reported in ppm downfield from the methyl carbon of the internal sulfonate, as previously described.[14] The spectra of T500 (20 mg) in neutral and alkaline solutions were similarly recorded with 116,000 and 122,000 scans. The difference spectra between the spectra of the acceptor and those of the acceptor products were also recorded on the spectrometer.[8] The assignments for the ^{13}C NMR signals of the alkaline and neutral T500 and the acceptor products were deduced from the assignments for glucans,[15] isomaltooligosaccharides,[14] and nigerooligosaccharides.[8] Based on these assignments, the various residues of the acceptor and the products were quantitatively estimated from the areas of the signals, isolated from each other if possible, or by the subtraction of isolated signal areas from overlapped ones.[15]

Isomaltopentaose (I_5) acceptor products were formed as just described, except for the addition of 100 mM (Glc equiv) I_5 (16.6 mg) instead of T500. The products were gel-filtered on a 100 mL (wet vol) column of Bio-Gel P-2 (Bio-Rad) equilibrated with distilled water. The yields after lyophilization were 11.3–13.0 mg. The ^{13}C NMR spectra of I_5 and its acceptor products were recorded with 73,000–127,000 scans, their difference spectra recorded, the signals assigned, and the residues quantitatively estimated, as before.

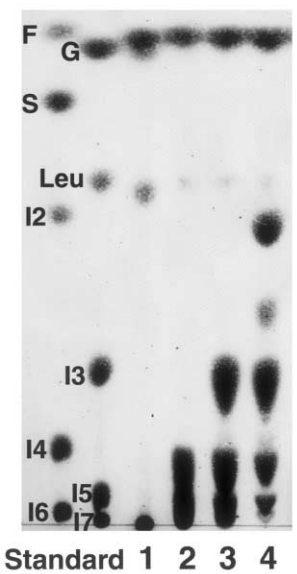

Fig. 1. TLC of acceptor reaction products of GTF-I on dextran T500 and isomaltooligosaccharides. Standard sugars: F, fructose; G, glucose; S, sucrose; Leu, leucrose; I_2–I_7, isomaltooligosaccharides of dp 2–7. Reaction mixtures containing 1 μM GTF-I, 100 mM sucrose, and 100 mM (Glc equiv) acceptors were incubated for 1 h and 2 μL of each mixture was chromatographed. Acceptors: lane 1, T500; lane 2–4, I_4–I_2.

The [U-^{13}C]dextran acceptor product was formed for 1 h in 1 mL of the buffer containing 1 μM GTF-I, 100 mM unlabeled sucrose and 10 mM (Glc equiv) [U-^{13}C] dextran (1.6 mg). The acceptor product (5.3 mg), after four washes and lyophilization, was dissolved in 0.4 mL of the alkaline solution. The spectra of [U-^{13}C]dextran and its acceptor product were recorded with 40,000 and 35,000 scans, the signals assigned, and the residues quantitatively estimated as before, taking special account of the content (1.1%) of ^{13}C at natural abundance.

3. Results

Acceptor products of S. sobrinus *sucrose glucosyltransferase GTF-I on dextran and isomaltooligosaccharides, as revealed by TLC.*— When dextran T500 was added at the same molarity (Glc equiv) as that (100 mM) of sucrose, GTF-I transferred Glc from sucrose mostly to T500 to give a product remaining at the origin (Fig. 1, lane 1), partly to water to give free glucose and fructose, and also partly to the 5-hydroxyl group of the resultant fructose to give leucrose (lane 1). When isomaltooligosaccharides of dp 4–2 (I_4–I_2) were added instead of T500, a series of higher isomaltooligosaccharides was produced (lanes 2–4) plus a small amount of an unknown trimer (lane 4), indicating that, when added at the same molarity (Glc equiv), isomaltooligosaccharides were better acceptors for Glc from sucrose than its polymer T500. In this respect, this dextran T500 (M_r 465 kDa) was composed of $\sim$63 chains of 43 α-(1→6)-linked Glc residues, which linked each other at branch points, as analyzed by ^{13}C NMR (data not shown). Thus, the concentrations of the nonreducing-end Glc of T500, I_4, I_3, and I_2 were 2.3, 25, 33 and 50 mM, respectively. Therefore, the difference in acceptor effectiveness between T500 and I_4–I_2 in this experiment might be due to the differ-

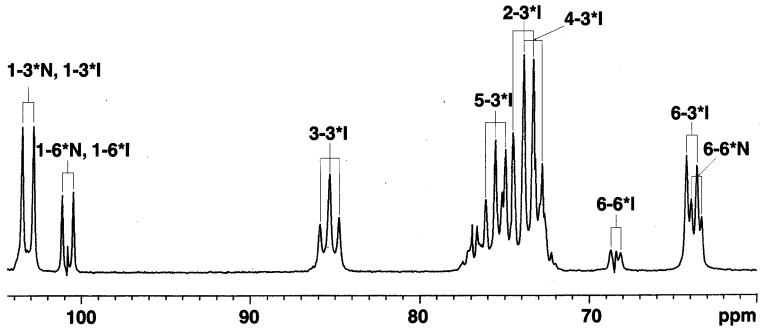

Fig. 2. ^{13}C NMR spectrum of the [U-^{13}C]Glc residues (*G) transferred from [U-^{13}C]sucrose to the T500 acceptor by GTF-I. This is the difference spectrum of the spectrum of T500 subtracted from that of the acceptor product in 0.5 M NaOH. 1-3*I–6-3*I represent the C-1–C-6 of the internal α-(1→3)-linked *G; 1-3*N, the C-1 of the nonreducing-end α-(1→3)-linked *G; 1-6*N and 6-6*N, the C-1 and C-6 of the nonreducing-end α-(1→6)-linked *G; 1-6*I and 6-6*I, the C-1 and C-6 of the internal α-(1→6)-linked *G.

ence in concentration of the nonreducing-end Glc.

Transfer of [U-^{13}C]Glc by GTF-I to T500, as revealed by NMR.—[U-^{13}C]Sucrose was used to distinguish the transferred Glc from the Glc of T500. When [U-^{13}C]sucrose (10 mM) was one tenth of the concentration (100 mM Glc equiv) of T500, 7.2 mM (72%) of the [U-^{13}C]Glc was transferred to T500, as estimated from the singlet signals for the Glc of T500 and the split signals for the [U-^{13}C]Glc transferred. The signals of the difference spectrum (Fig. 2), obtained by subtracting the T500 spectrum from its product spectrum, are for the [U-^{13}C]Glc transferred. The signals for the internal α-(1→3)-linked [U-^{13}C]Glc appeared as doublets (C-1, 103.2 ^1J 45.9; C-6, 63.9 ppm ^1J 42.5 Hz) and as triplets (C-2, 73.9 ^1J 39.9; C-3, 85.3 ^1J 37.4; C-4, 73.3 ^1J 38.1; C-S, 75.5 ppm ^1J 39.4 Hz) in alkaline solution. Some isolated or overlapped signals for the nonreducing-end α-(1→6)-linked [U-^{13}C]Glc (C-1, 100.8 ^1J 45.4; C-6, 63.6 ppm ^1J 41.8 Hz), for the internal α-(1→6)-linked [U-^{13}C]Glc (C-1, 100.8 ^1J 45.4; C-6, 68.4 ppm ^1J 35.4 Hz), and for the nonreducing-end α-(1→3)-linked [U-^{13}C]Glc (C-1, 103.2 ppm ^1J 45.9 Hz) were also detected. This observation indicated that the elongation of the α-(1→3)-linked chain was already overwhelming at this concentration of sucrose. Therefore, in order to monitor the transfer mechanism at an earlier stage, the reaction was conducted similarly at lower concentrations (1–7 mM) of [U-^{13}C]sucrose and each type of Glc transferred to T500 was estimated quantitatively (Fig. 3). At 1–10 mM [U-^{13}C]sucrose, ~20% of the [U-^{13}C]Glc was transferred to the nonreducing-end Glc of T500 by an α-(1→6) linkage, resulting in the formation of a new nonreducing-end α-(1→6)-linked [U-^{13}C]Glc and internal α-(1→6)-linked [U-^{13}C]Glc. The amount of nonreducing-end α-(1→3)-linked [U-^{13}C]Glc reached a maximum (0.27 mM) at 4 mM [U-^{13}C]sucrose and remained almost constant up to 10 mM [U-^{13}C]sucrose. Meanwhile, the internal α-(1→3)-linked [U-^{13}C]Glc increased markedly with increasing concentration of [U-^{13}C]sucrose, and the average number of α-(1→3)-linked [U-^{13}C]Glc residues per branched chain reached 18 at 10 mM [U-^{13}C]sucrose. This result suggested that the

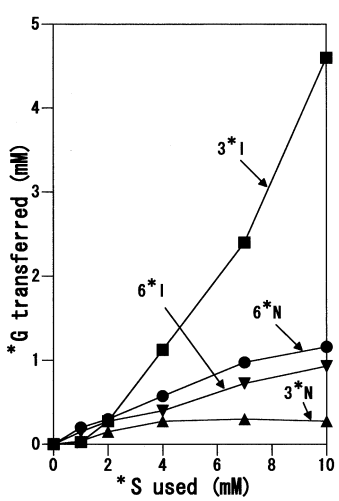

Fig. 3. Linkage of the [U-^{13}C]Glc residues (*G) transferred from [U-^{13}C]sucrose (*S) to 100 mM (Glc equiv) T500. 3*I, the internal α-(1→3)-linked *G; 6*N, the nonreducing-end α-(1→6)-linked *G; 6*I, the internal α-(1→6)-linked *G; 3*N, the nonreducing-end α-(1→3)-linked *G.

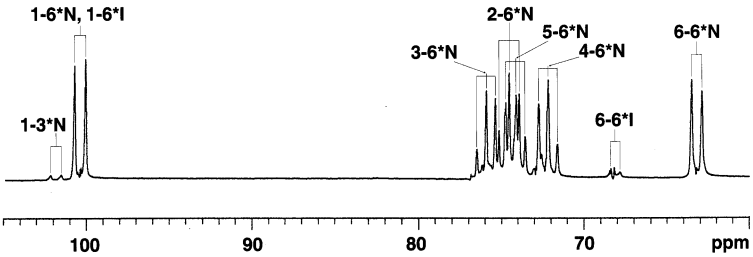

Fig. 4. ^{13}C NMR spectrum of the [U-^{13}C]Glc residues (*G) transferred from [U-^{13}C]sucrose to the isomaltopentaose (I_5) acceptor by GTF-I. This is the difference spectrum of the spectrum of I_5 subtracted from that of the acceptor product in neutral solution. 1-6*N–6*N, the C-1–C-6 of the nonreducing-end α-(1→6)-linked *G; 1-3*N, the C-1 of the nonreducing-end α-(1→3)-linked *G; 1-6*I and 6-6*I, the C-1 and C-6 of the internal α-(1→6)-linked *G.

transfer reaction was accelerated by the elongating chains composed of more than 4 or 5 α-(1→3)-linked [U-^{13}C]Glc residues.

Transfer of [U-^{13}C]Glc by GTF-I to I_5.—The foregoing data revealed that the major acceptor-site on T500 was the nonreducing-end α-(1→6)-linked Glc. In order to confirm this presumption, isomaltopentaose (I_5), a straight α-(1→6)-linked Glc chain, was used as the acceptor instead of T500, and the acceptor product was analyzed by NMR, as already described. When [U-^{13}C]sucrose (10 mM) was again one tenth of the concentration (100 mM Glc equiv) of I_5, ~7 mM (70%) of the [U-^{13}C]Glc was transferred to I_5. The difference spectrum in neutral solution (Fig. 4) was obtained by subtracting the spectrum of I_5 from that of the acceptor product. The major signals are for the nonreducing-end α-(1→6)-linked [U-^{13}C]Glc (C-1, 100.4 ^1J 44.9; C-2, 74.5 ^1J 41.1; C-3, 75.9 ^1J 37.2; C-4, 72.2 ^1J 39.6; C-5, 74.2 ^1J 39.9; C-6, 63.2 ppm ^1J 42.5 Hz). The minor signals are for the nonreducing-end α-(1→3)-linked [U-^{13}C]Glc (C-1, 101.8 ppm ^1J 43.7 Hz) and for the internal α-(1→6)-linked [U-^{13}C]Glc (C-6, 68.1 ppm ^1J 37.1 Hz). The doublet signals (C-1α, 94.9 ppm; C-1β, 98.8 ppm) for the α and β forms of the reducing-end [U-^{13}C]Glc and the triplet signal for the α-1,3,6-linked [U-^{13}C]Glc (C-3, 85.0 ppm ^1J 37.7 Hz)[8] were never detected in Fig. 4. At 1–10 mM [U-^{13}C]sucrose (Fig. 5), roughly 90% of the [U-^{13}C]Glc transferred was at the 6-hydroxyl group of the nonreducing-end Glc of I_5 and 7% was at the 6-hydroxyl group of the [U-^{13}C]Glc α-(1→6)-linked to I_5. The rest (3%) was at the 3-hydroxyl group of the nonreducing-end Glc of I_5.

[U-^{13}C]Dextran and the branch-forming ability of GTF-I on the [U-^{13}C]dextran.—The [U-^{13}C]dextran synthesized from [U-^{13}C]sucrose by a *S. bovis* dextransucrase had M_r higher than 2000 kDa. In neutral solution, the major signals (Fig. 6(A)) are for the internal α-(1→6)-linked [U-^{13}C]Glc (96.5%) (C-1, 100.4 ^1J 45.6; C-2, 74.1 ^1J 41.1; C-3, 76.2 ^1J 37.1; C-4, 72.2 ^1J 37.0; C-5, 73.0 ^1J 38.6, C-6, 68.2 ppm ^1J 40.3 Hz). The minor signals are for the α-1,3,6-linked [U-^{13}C]Glc (1.1%) (C-3, 83.3 ppm ^1J 37.0 Hz), for the [U-^{13}C]Glc α-(1→3)-linked to the branched [U-^{13}C]Glc (1.1%) (C-1, 102.0 ppm ^1J 43.0 Hz) and for the nonreducing-end α-(1→6)-linked [U-^{13}C]Glc (1.3%) (C-6, 63.1 ppm ^1J 41.7 Hz). The doublet signals (C-1α, 94.9 ppm; C-1β, 98.8 ppm) for the α and β forms of the reducing-end [U-^{13}C]Glc were never detected, as not visible

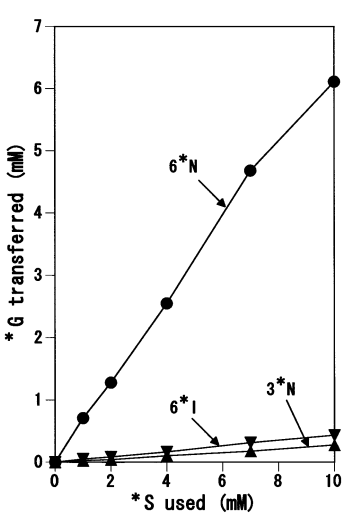

Fig. 5. Linkage of the [U-^{13}C]Glc residues (*G) transferred from [U-^{13}C]sucrose (*S) to 100 mM (Glc equiv) I_5. 6*N, the nonreducing-end α-(1→6)-linked *G; 6*1, the internal α-(1→6)-linked *G; 3*N, the nonreducing-end α-(1→3)-linked *G.

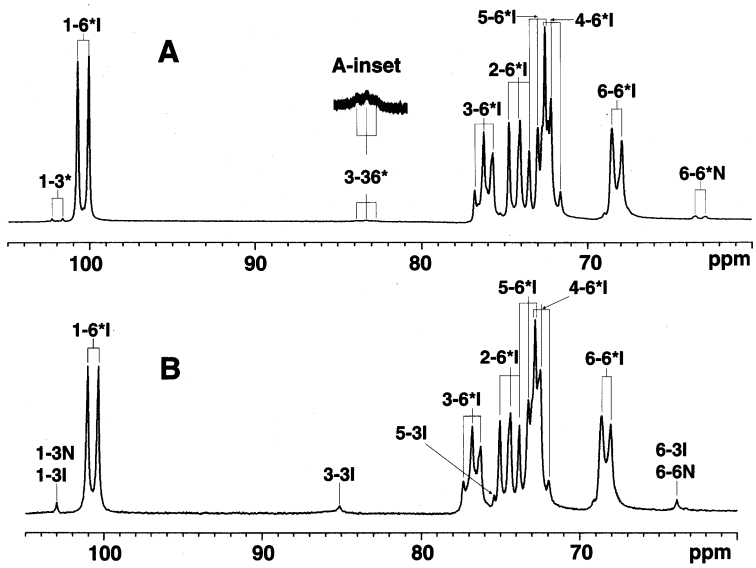

Fig. 6. ^{13}C NMR spectra of [U-^{13}C]dextran (*D) and the resulting product from *D and unlabeled sucrose. (A) The *D in neutral solution, which was de novo synthesized from [U-^{13}C]sucrose by a *S. bovis* dextransucrase. (A, inset), signals of ten times-higher sensitivity. (B) The resulting product in alkaline solution. 1-6*I–6-6*I, the C-1–C-6 of the internal α-(1→6)-linked [U-^{13}C]Glc residues (*G); 3-36*, the C-3 of the α-1,3,6-branched *G; 1-3*, the C-1 of the *G α-(1→3)-linked to the 3-hydroxyl group of the branched *G; 6-6*N, the C-6 of the nonreducing-end α-(1→6)-linked *G; 1-3I, 3-3I, 5-3I and 6-3I, the C-1, C-3, C-5 and C-6 of the internal α-(1→3)-linked unlabeled Glc residue (G) transferred; 1-3N, the C-1 of the nonreducing-end α-(1→3)-linked G; 6-6N, the C-6 of the nonreducing-end α-(1→6)-linked G.

in Fig. 4. Based on these data, this [U-^{13}C]dextran was estimated to be a macromolecule higher than 2000 kDa, composed of more than 150 chains having an average of 77 contiguously α-(1→6)-linked [U-^{13}C]Glc residues linked to each other at the branch points.

The acceptor reaction was conducted by adding an excess (100 mM) of unlabeled sucrose to the [U-^{13}C]dextran (10 mM Glc equiv) in order to detect branch formation by GTF-I on the chains of contiguously α-(1→6)-linked [U-^{13}C]Glc. Approximately 25 mM (25%) of the unlabeled Glc was transferred to the [U-^{13}C]dextran, as measured by the phenol–sulfuric method. The major split signals of the acceptor product in alkaline solution (Fig. 6(B)) are for the internal α-(1→6)-linked [U-^{13}C]Glc of the [U-^{13}C]dextran (C-1, 100.7 ^1J 46.4; C-2, 74.4 ^1J 41.9; C-3, 76.8 ^1J 36.1; C-4, 72.4 ^1J 30.5; C-5, 73.3 ^1J 32.7, C-6, 68.7 ppm ^1J 38.6 Hz). The minor singlet signals are for the internal α-(1→3)-linked Glc transferred (25 mM) (C-1, 103.0; C-3, 85.1; C-6, 63.8 ppm) and for the nonreducing-end α-(1→6)-linked Glc transferred (6 mM) (C-6, 63.3 ppm). Any increase in strength of the triplet signal for the α-1,3,6-linked [U-^{13}C]Glc (C-3, 85.0 ppm ^1J 37.7 Hz)[8] was not detected. This observation indicated that the unlabeled Glc from sucrose was not transferred at the 3-hydroxyl group of the contiguously α-(1→6)-linked [U-^{13}C]Glc of the [U-^{13}C]dextran acceptor, confirming that GTF-I could not form new α-1,3,6-linked branch points on dextran chains.

4. Discussion

In this study, dextran T500 was used as one of the most effective dextran acceptors for GTF-I.[6] Isomaltopentaose was also used as one of the contiguously α-(1→6)-linked oligosaccharides. Linkage structures for the [U-^{13}C]chains formed by GTF-I were estimated based on ppm shift values and the $^{13}C–^{13}C$ one-bond coupling constant (^1J) for C-1–C-6 of the [U-^{13}C]Glc,[8,15,21,22] specifically by using [U-^{13}C]sucrose. Most of the [U-^{13}C]Glc from 1 mM [U-^{13}C]sucrose was found to become α-(1→6)-linked to the non-reducing-end Glc (~2.3 mM) of 100 mM (Glc equiv) T500. Most of the [U-^{13}C]Glc from 10 mM [U-^{13}C]sucrose is also α-(1→6)-linked to the non-reducing-end Glc (20 mM) of 100

mM (Glc equiv) isomaltopentaose. The rest of the [U-^{13}C]Glc was found to be also α-(1→3)-linked to the non-reducing-end Glc of these isomaltooligosaccharides. As in this study, Fu and Robyt[16] demonstrated clearly by TLC that the GFT-I of *S. sobrinus* transferred Glc from [U-^{14}C]sucrose to the non-reducing-end Glc of panose (6^2-α-glucosyl maltose) to give 6^2-α-isomaltosyl maltose and thus, forming α-(1→6) linkage. They also observed 6^2-α-nigerosylmaltose as a minor product, indicating the formation of α-(1→3) linkages. It could be inferred from these observations that the minor product (Fig. 1, lane 4) was 3^2-α-glucosylisomaltose. Transfer of [U-^{13}C]Glc to the reducing-end of the acceptors did not take place, since the doublet signals for the α and β forms of the reducing-end [U-^{13}C]Glc were never detected in any of the ^{13}C NMR spectra in neutral solution.

The transfer of [U-^{13}C]Glc to the 3-hydroxyl group of the internal Glc of T500 or isomaltopentaose was also not detectable by NMR (Figs. 2 and 4). In order to confirm this, [U-^{13}C]dextran was specifically synthesized from [U-^{13}C]sucrose by a *S. bovis* dextransucrase, and the Glc from normal sucrose was transferred to the [U-^{13}C]dextran (Fig. 6(A and B)). If one branch point was formed by GTF-I at every ten Glc residues of dextran, as suggested,[4] 10% of the internal α-(1→6)-linked [U-^{13}C]Glc (Fig. 6, 6*I) of [U-^{13}C]dextran would shift to the α-1,3,6-branched [U-^{13}C]Glc (36*). Furthermore, if α-(1→3)-glucan chains were transferred at various positions along dextran as suggested,[10] a shift of several % would also occur. However, such a shift was never detected (less than 0.1%) in this study, indicating that there was no direct branch-forming activity of GTF-I, as suggested.[9] In this regard, T500 dextran from *Leuconostoc mesenteroides* B-512F, the dextran from *S. bovis* 9809, and the dextran synthesized by *S. sobrinus* GTF-S2 resemble each other, being composed of mainly α-(1→6) linkages with some or several percentage of α-(1→3) linkages or α-1,3,6-branches.[3,11] In contrast, the dextran synthesized by *S. sobrinus* GTF-S1 is highly branched [64% of α-(1→6) linkage and 36% of α-(1→3) linkages]. Therefore, the branch-forming activity among *S. sobrinus* extracellular enzymes may be attributed mainly to the GTF-S1.[17,18]

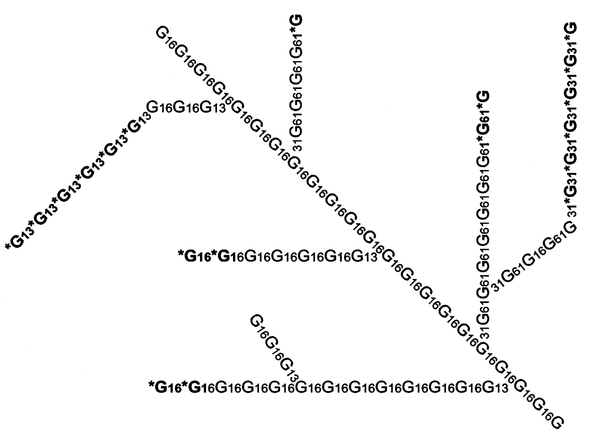

Fig. 7. Schematic structure for the product resulting from an acceptor dextran and a limited amount of [U-^{13}C]sucrose. GTF-I transferred the ^{13}C-labeled glucosyl residue (***G**) to the nonreducing-end glucosyl residue (G) of dextran, mostly by α-(1→6) linkages and partially by α-(1→3) linkages, and then preferentially elongated the α-(1→3)-linked chains.

The observations in this study may be interpreted as follows. Dextran T500[19] and *S. bovis* [U-^{13}C]dextran have structures of many α-(1→6)-linked Glc chains attached at α-(1→3)-branch linkages and the non-reducing-ends of Glc are abundant (Fig. 7), as compared to the number of GTF-I (130–2300 per one GTF-I molecule). GTF-I initially transferred Glc to the non-reducing ends of the same number, mostly by α-(1→6) linkages and partially by α-(1→3) linkages (Fig. 7). Kaseda et al.[20] observed, using total internal reflection fluorescence, that the interaction time, during which GTF-I traveled from one dextran to another, was 75 ms. The transfer time when GTF-I transferred Glc from sucrose to dextran was 40 ms. Therefore, after transferring one Glc or two Glc residues successively, GTF-I dissociates from the dextran. Then the GTF-I associates again with an other dextran or the elongated dextran, and the transfer and dissociation is repeated until the terminal is elongated by more than four or five α-(1→3)-linked Glc residues. Thereafter, transfer to the α-(1→3)-linked terminal was particularly accelerated (Figs. 3 and 7), just as the nigerooligosaccharides higher than dp 5 accelerated the GTF-I activity.[8]

The use of [U-^{13}C]sucrose was again successful for the analysis of Glc transferred to acceptors (Figs. 2 and 4), as noted in our previous study.[8] In this study, [U-^{13}C]dextran was also specifically used in an effort to detect the shift of internal α-(1→6)-linked Glc of dextran to the branched Glc (Fig. 6). The values of the ^{13}C–^{13}C one-bond coupling constant (^{1}J) are presented for the C-1–C-6 bond of the α-(1→3)-linked [U-^{13}C]Glc and of the non-reducing-end [U-^{13}C]Glc,[8] and also for the C-1–C-6 of the α-(1→6)-linked [U-^{13}C]Glc and of the non-reducing-end [U-^{13}C]Glc in this study. These values are expected to aid in the assignment of split signals in future studies using [U^{13}-C]sucrose and [U-^{13}C]glucan.

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