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Formation of α-(1 → 6), α-(1 → 3), and α-(1 → 2) glycosidic linkages by dextransucrase from *Streptococcus sanguis* in acceptor-dependent reactions *

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ABSTRACT

Dextransucrase from *Streptococcus sanguis* 10558 was found to synthesize α-(1 → 6), α-(1 → 3), and α-(1 → 2) linkages during an acceptor-dependent glucosyl transfer reaction. Normally, new glucosyl residues are added at C-6 of monosaccharide acceptors. However, sugars blocked at C-6 also can serve as good acceptors. The disaccharide and trisaccharide products formed when methyl 6-bromo-6-deoxy-α-D-glucopyranoside was used as acceptor were isolated and characterized. Both were found to contain only α-(1 → 3) glycosidic bonds. This supports the hypothesis that when C-6 is blocked the acceptor binds to the enzyme in a flipped orientation, resulting in an approximate exchange in space of the C-3 and C-6, thereby putting C-3 adjacent to the active site. The second α-(1 → 3) links in the trisaccharide are formed by a single-chain mechanism without release of the intermediate disaccharide. With maltose as acceptor, new glucosyl residues are added at C-6′. However, if that position is blocked with a bromine atom, the resulting compound, 6′-bromo-6′-deoxy-maltose, can still serve as an acceptor. The product in this case was isolated and characterized. The new glycosidic link was found to be α-(1 → 2).

INTRODUCTION

Dextransucrase (EC 2.4.1.5), produced extracellularly by bacterial species of *Leuconostoc, Lactobacillus*, and *Streptococcus*, is a glucosyl transferase that catalyzes the formation of dextran by transfering the glucosyl portion of sucrose to a growing glucose homopolymer. The dextran is a major component of dental plaque which anchors the bacteria close to the tooth surface and also serves as a stable matrix for trapping nutrients needed by the bacteria for their metabolism[1,2]. Fructose, which is a byproduct of the dextransucrase reaction, is metabolised by the bacteria to produce organic acids. The plaque serves as diffusional barrier for these acids which concentrate on the tooth surface and degrade the tooth enamel[3]. Dextrans are compounds of diverse structure depending on the bacterial strains

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producing them. *S. mutans* produces two types of glucosyltransferases, GTF-S and GTF-I, which catalyze the formation of α-(1 → 6) and α-(1 → 3) linked dextran, respectively[4]. The presence of dextran-containing sequences of α-(1 → 6) linked D-glucose residues stimulates the activity of GTF-I[4]. *S. sanguis* produces only one type of dextran whose structure has been characterized as follows: 53% of the glucose residues are substituted at C-6 and 15% at C-3, while 16% are located at branch points, and 16% are terminal, nonreducing end residues[5]. The polymerization reaction takes place by the transfer of glucose from sucrose to the reducing end of the growing dextran chain by an insertion mechanism[6,7]. It has been shown that the active site of α-(1 → 3) glucan synthase from *S. mutans* AHT (serotype g) has two distinct sites for sucrose hydrolysis and glucosyl-transfer activities[8].

Besides polymer formation, dextransucrase can also catalyze the transfer of glucose residues to added acceptor substrates. In contrast to the narrow donor-substrate specificity[9–11], the acceptor-substrate specificity is rather broad[12,13]. In the presence of acceptors, addition of new glucose units takes place at the nonreducing end[14], whereas in the polymerization reaction addition is at the reducing end[7,15]. With methyl α-D-glucopyranoside as acceptor, a new glucose unit is added at position-6[14,16,17]. However, we have reported earlier that if position-6 is blocked as in 6-deoxy or 6-deoxy-6-halo derivatives, the compounds still serve as effective acceptor substrates for dextransucrase[13]. In this respect dextransucrase from *S. sanguis* is different from *L. mesenteroides* B-512 FM dextransucrase that cannot use the 6-blocked derivatives as acceptors[18]. The formation of products from the analogues blocked at position-6 was unexpected, and so it was of interest to determine the structures of the products formed. In the present work we provide evidence that when C-6 is blocked, a new glucosyl residue is added at C-3 to form an α-(1 → 3) linkage.

EXPERIMENTAL

Materials. —Dextransucrase was purified from *S. sanguis* (ATCC 10558) according to method of Grahame and Mayer[19]. Isomaltose, maltose, methyl α-D-glucopyranoside, and invertase were from Sigma Chemical Company (St. Louis, MO), silica gel and plastic-backed silica gel TLC plates were from E. Merck (Gibbstown, NJ), Whatman 1 and 3MM chromatography papers were from Whatman, Inc. (Clifton, NJ), carbon tetrabromide and deuterated solvents were from Aldrich Chemical Company (Milwaukee, WI), *N*-bromosuccinimide was from Fisher Scientific Company (Fair Lawn, NJ), [*U*-^{14}C]sucrose and [*glucose*-^{14}C(U)]sucrose were from New England Nuclear Corporation (Boston, MA) and methyl α-D-[U-^{14}C]glucopyranoside was from Amersham (Arlington Heights, IL). Nigerose was kindly provided by I. Goldstein of The University of Michigan, Ann Arbor, MI. All other materials were of reagent grade and available from common sources.

Methyl 6-bromo-6-deoxy-α-D-glucopyranoside was synthesized, according to the method of Anisuzzaman and Whistler[20], by reacting methyl α-D-glucopyranoside

with carbon tetrabromide and triphenyl phosphine in pyridine. The product was purified by cystallization twice from an ethanol–hexane mixture and characterized by melting point and NMR spectroscopy. It was made sure that the product was free of (bromomethyl)triphenylphosphonium bromide, which is a byproduct of the reaction and a potent inhibitor of dextransucrase[11].

6′-Bromomaltose was synthesized by method of Takeo and Shinmitsu[21]. 4′,6′-*O*-Benzylidene maltose hexaacetate was first made from maltose. It was then brominated with *N*-bromosuccinimide to give 4′-*O*-benzoyl-6′-bromo-6′-deoxymaltose hexaacetate. The benzoyl and acetate groups were removed in the presence of sodium methoxide to give the title compound. The product was finally purified by a silica gel column and characterized by its NMR spectrum.

Methods. —NMR spectoscopic methods. ^1H and ^{13}C NMR spectra were recorded on a Bruker WP-200 FT spectrometer. Sugars were dissolved in D_2O or $CDCl_3$ with Me_4Si or acetone as internal reference. For ^1H NMR spectroscopy in D_2O, the HDO peak was used as reference and assigned at 4.63 ppm. All chemical shifts are expressed in ppm downfield from Me_4Si. Peak assignments were made by comparison with published[23,26] spectra of reference sugars (maltose, isomaltose, and linear and branched trisaccharides). Assignments of peaks relavant to this study are discussed later.

Chromatographic procedures. —Descending paper chromatography was conducted in one of the following solvent systems: (*I*) 6:1:3 propanol–EtOAc–water and (*II*) 9:1:1 butanone–acetic acid–boric acid satd water. Standard sugars used as markers in separate lanes were visualized by staining with silver nitrate–NaOH[22]. Radioactive chromatograms were cut into 1-cm strips, and their radioactivity was counted in a liquid scintillation counter.

Assay of acceptor activity. —Reactions were carried out in mixtures containing acceptor (50 mM), [*glucose* $^{14}C(U)$]sucrose (67 mM, 0.04 μCi), and 0.05 units of enzyme in a total volume of 30 μL, at 37°C for 60 min, after which the mixtures were spotted on Whatman 1 paper. The chromatogram containing 6′-bromo-6′-deoxymaltose was developed overnight in *II*. Others were developed in *I*. During that time, sucrose moved 21 cm in *I* and 7 cm in *II*. The chromatograms were dried, cut into 1-cm strips, and their radioactivity was counted. Any glucose produced by enzymic hydrolysis of sucrose was not counted as an acceptor product. Radioactivity co-migrating with sucrose, when the bromo-derivatives were used as acceptors, was eluted from the chromatogram, treated with 4 units of invertase, and spotted on paper and rechromatographed in *II*. An invertase-resistant product was considered as an acceptor product.

Preparative synthesis of acceptor products. —A. *With methyl α-D-glucopyranoside.* The reaction mixture contained 17 units of enzyme, sucrose (200 mM), and acceptor (140 mM) in a total volume of 9 mL. It was allowed to react at 37°C overnight.

B. With maltose. Enzyme (7 units), sucrose (100 mM), and acceptor (140 mM) in a total volume of 5.6 mL were reacted overnight at 37°C.

C. With methyl 6-bromo-6-deoxy-α-D-glucopyranoside. Enzyme (1.3 units), acceptor (700 mM), and sucrose (5.4 mM) in a total volume of 0.9 mL were reacted at 37°C. More sucrose was added to it at regular intervals as follows: 5 μmol every 10 min for 3 h, then 2 μmol every 10 min for 3 h, then 1 μmol every 10 min for 2 h. The final volume was 1.6 mL. The reaction was allowed to continue overnight.

D. With 6'-bromo-6'-deoxymaltose. Enzyme (5 units), sucrose (50 mM), and acceptor (200 mM) in a total volume of 2 mL were reacted at 37°C. Sucrose (100 μmol) was added every hour for 3 h. The final volume was 3.5 mL. The reaction was continued for another 4 h, after which time it was treated with 60 units of invertase in a total volume of 3.6 mL at 55°C for 30 min.

The mixtures were spotted at the origin of Whatman 3MM paper chromatograms (1-cm length for every 40 μL). The chromatograms were developed in *I* until the solvent front had moved 50 cm. In the case of 6'-bromo-6'-deoxymaltose, the chromatogram was developed in *II* for 36 h. It was dried and developed for a further 36 h. In *I* the acceptor product co-migrates with sucrose.

In each case a parallel reaction, which was developed separately, was carried out on 0.01-scale with [U^{14}C]sucrose (33 μCi/nmol). By counting the radioactivity, the positions of the products of interest were determined and the corresponding products with the unlabeled sucrose were eluted from the paper chromatogram with water, filtered, lyophilized, and prepared for NMR analysis.

RESULTS

Sugars blocked at C-6 are good acceptors for dextransucrase[13]. To determine the structures of the products formed, preparative-scale reactions were carried out. When the acceptor ability of methyl 6-bromo-6-deoxy-α-D-glucopyranoside was measured under standard assay conditions, a major disaccharide and a small amount of trisaccharide were produced. The disaccharide-to-trisaccharide ratio was 55:1 (ref. 13). In order to increase the yield of the trisaccharide, a preparative-scale reaction was carried out as described in Experimental, such that the ratio of acceptor-to-donor substrates was very high throughout the reaction. The result (Table I, line 5) shows that the proportion of trisaccharide was significantly increased (disaccharide-to-trisaccharide ratio 2:1). Following paper chromatographic purification, the ^{13}C and ^{1}H NMR spectra were obtained for the disaccharide products from the two acceptors, methyl α-D-glucopyranoside and methyl 6-bromo-6-deoxy-α-D-glucopyranoside (Figs. 1 and 2). The ^{13}C NMR spectrum of the disaccharide product formed from methyl α-D-glucopyranoside (Fig. 1A) gave two equivalent peaks with chemical shifts of 61.4 and 66.5 ppm, which were assigned to unsubstituted C-6 and α-(1 → 6) substituted C-6, respectively[23]. This, and the appearance of peak at 98.8 ppm [C-1, α-(1 → 6)], indicates that the structure of the disaccharide product is methyl 6-*O*-α-D-glucopyranosyl-α-D-glucopyranoside (i.e., α-methyl isomaltose). Similar conclusions can be drawn from the ^{1}H NMR spectrum (Fig. 1B), where the anomeric protons are the most sensitive

TABLE I

Activities of some acceptors [a]

Line	Acceptor	Polymer formed (% dpm)	Acceptor products		Total acceptor product (% dpm)	Total product (% dpm)
			Peak position (R_s)	(% dpm)		
1	None	77.1				77.1
2	Maltose	2.8	0.05–0.30	44.1	71.8	74.6
			0.30–0.45	18.7		
			0.45–0.65	9.0		
3	Isomaltose	7.5	0.04–0.17	15.2	89.1	96.6
			0.17–0.26	19.7		
			0.26–0.39	31.9		
			0.39–0.57	22.3		
4	Nigerose	25.6	0.05–0.24	32.2	41.3	66.9
			0.24–0.33	3.4		
			0.33–0.48	2.3		
			0.52–0.67	0.9		
			0.67–0.86	2.5		
5	6′-Bromo-6′-deoxymaltose	22.3	0.25–0.88	15.0	15.0	37.3
6	Methyl 6-bromo-6-deoxy-α-D-glucopyranoside	44.6	1.93–2.20	11.7	34.5	79.1
			2.20–2.53	22.8		

[a] For lines 1–5 and line 6, reaction conditions were as described in Experimental: assay of acceptor activity and preparative synthesis of acceptor products, respectively. Peak positions are shown relative to sucrose (R_s). Position of sucrose was at 8 cm for line 5, 15 cm for line 6, and 21 cm for the others. For line 6, only counts in the glucose portion of sucrose have been considered for the calculation of % dpm, although uniformly labeled sucrose was used.

indicators of linkage types. The doublet at 4.9 ppm reflects the presence of an α-(1 → 6) bond[24]. The higher homologs were also isolated, and their ^{13}C and ^{1}H NMR spectra (not shown) revealed the presence of only α-(1 → 6) linkages. A comparison with the ^{13}C NMR spectrum of the disaccharide product formed from methyl 6-bromo-6-deoxy-α-D-glucopyranoside (Fig. 2A) indicates that the peak at 66.5 ppm is missing, while a peak at 80.4 ppm appears. This has been assigned to C-3 in α-(1 → 3) glucosidic bonds[23]. This is also illustrated by the appearance of the peak at 100.0 ppm, which is the chemical shift of C-1 in the same linkage[23]. The doublet at 5.3 ppm in the ^{1}H NMR spectrum of the disaccharide from the 6-bromo derivative (Fig. 3A) represents α-(1 → 3) bonds[24]. It should be pointed out that there is a small doublet with a chemical shift at 5.3 ppm in the ^{1}H NMR spectrum of the disaccharide from methyl α-D-glucopyranoside (Fig. 1B), which indicates that a small proportion (< 2%) of α-(1 → 3) linkage is generated even when position 6 is available. We conclude from these studies that during the glucosyl-transfer reaction, the enzyme preferentially catalyzes the formation of

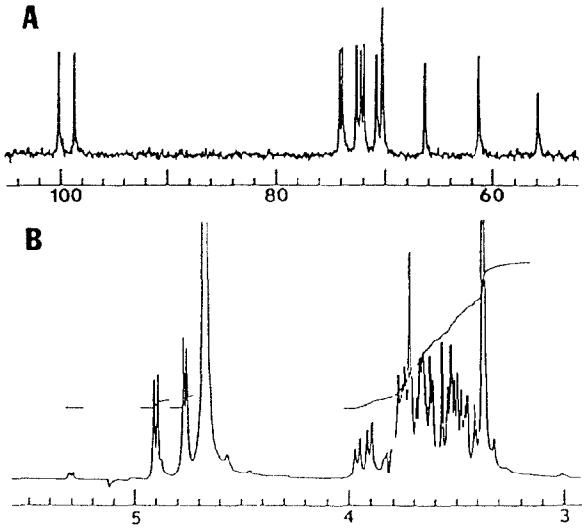

Fig. 1. NMR spectra of the disaccharide acceptor product formed by using methyl α-D-glucopyranoside as acceptor. (A) ^{13}C NMR spectrum, (B) ^{1}H NMR spectrum.

α-(1 → 6) bonds, but can also form an α-(1 → 3) bond, which is the exclusive linkage when the position 6 is blocked.

The trisaccharide product formed with the bromo derivative as acceptor was also eluted from the chromatogram and was characterized by its NMR spectra (Figs. 2B and 3B). Based on a similar analysis as in the case of the disaccharide, it can be said that the trisaccharide has two α-(1 → 3) linkages and no α-(1 → 6) linkage. In the ^{13}C NMR spectrum, the presence of new peaks at 81.1 and 100.1

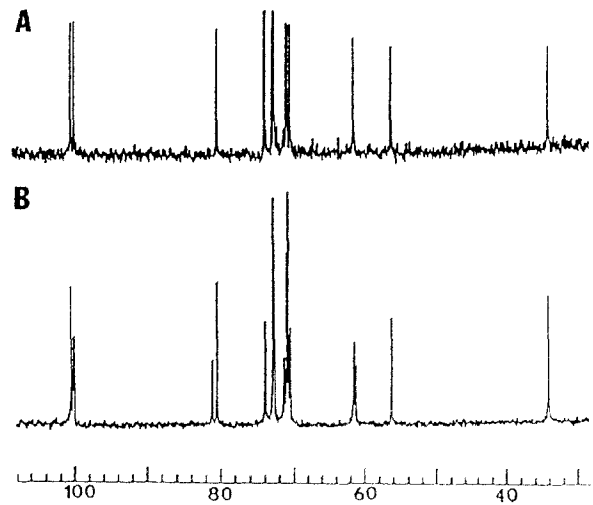

Fig. 2. ^{13}C NMR spectra of acceptor products formed by using methyl 6-bromo-6-deoxy-α-D-glucopyranoside as acceptor. (A) Disaccharide product, (B) trisaccharide product.

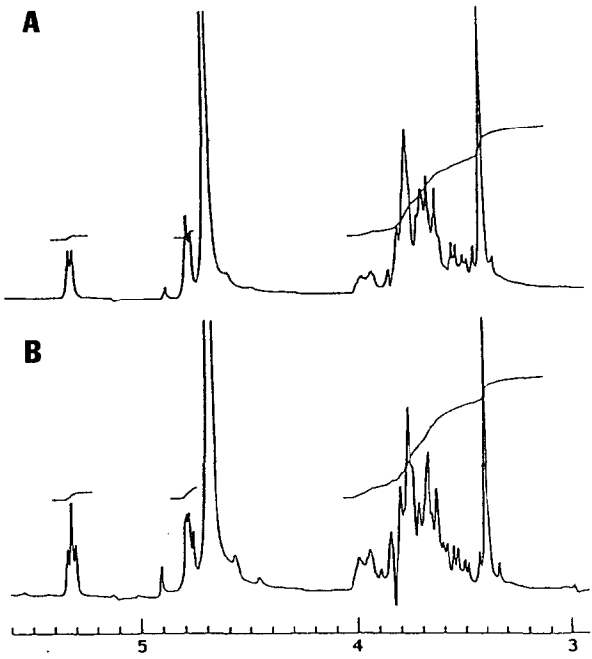

Fig. 3. ^{1}H NMR spectra of acceptor products formed by using methyl 6-bromo-6-deoxy-α-D-glucopyranoside as acceptor. (A) Disaccharide product, (B) trisaccharide product.

ppm indicate the second sugar linkage is also α-(1 → 3). This was confirmed by the doublet at 5.3 ppm in the ^{1}H NMR spectrum. The complete absence of the α-(1 → 6) bond is evident from the lack of peaks at 67 ppm in the ^{13}C NMR spectrum and at 4.9 ppm in the ^{1}H NMR spectrum.

Some disaccharides were also tested as acceptors. The results (Table I) show that 6′-bromo-6′-deoxymaltose is a poor acceptor compared to maltose, and it also inhibits total enzyme activity. In contrast, analogues of the monosaccharide, methyl α-D-glucopyranoside that are modified at position-6 do not inhibit polymerization or acceptor reaction[13]. 6′-Bromo-6′-deoxymaltose gives only one acceptor product. This is similar to a previous observation that monosaccharide analogues modified at position-6, the normal site of glucosyl transfer, also gave single acceptor products. Nigerose is different from other acceptors in that it produces oligosaccharides of higher degrees of polymerization, indicating that the acceptor products are also good acceptors.

With maltose as acceptor, new glucosyl residues are added at the C-6 of the nonreducing ends[25]. We have also confirmed this by isolating and characterizing the acceptor products using ^{1}H and ^{13}C NMR spectroscopy (results not shown). So if this position is blocked, as in 6′-bromo-6′-deoxymaltose, it may be expected that the compound cannot serve as an acceptor. However, Table I shows that 6′-bromo-6′-deoxymaltose does serve as an acceptor, though not as well as maltose. There is a single product formed that could be separated from sucrose in solvent system II,

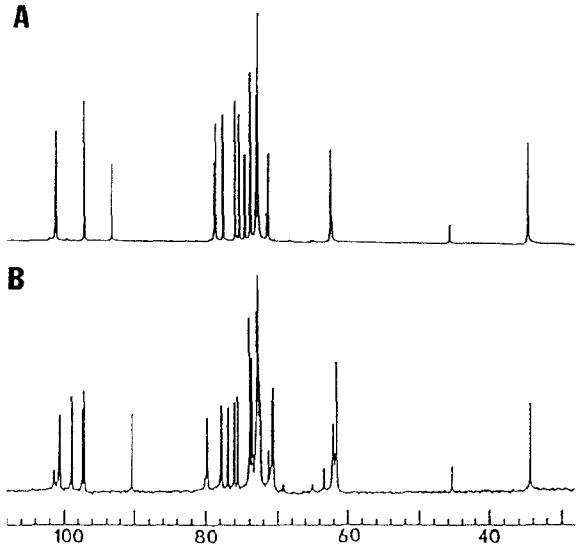

Fig. 4. ^{13}C NMR spectra of acceptor substrate and product. (A) 6′-Bromo-6′-deoxymaltose, (B) Trisaccharide acceptor product formed by using 6′-bromo-6′-deoxymaltose as acceptor.

but not in solvent system I. To determine the type of linkage, the reaction was carried out on a preparative scale, the product was isolated from the paper chromatogram and analysed by ^{13}C NMR spectroscopy (Fig. 4B). There is no peak at 67 ppm suggesting that there is no α-(1 → 6) linkage. Usui et al.[26] have studied the ^{13}C NMR spectra of various gluco-oligosaccharides. They pointed out that the chemical shifts of the α and β anomeric forms of all reducing sugars are similar in magnitude, and that the C-1 resonance of the β-form is shifted downfield by ~ 3.9 ppm from that of the α form except in the case of α-(1 → 2) linked glucobioses, which show C-1 signals at 90.4 ppm for the α anomer and 97.1 ppm for the β anomer. The C-1α peak has been shifted from 92.8 ppm in 6′-bromo-6′-deoxymaltose (Fig. 4A) to 90.2 ppm in the product (Fig. 4B), indicating that the new glucose residue is added at the C-2 of the reducing end as an α-(1 → 2) linkage. If it is added at the nonreducing end, the chemical shift of the reducing-end anomeric proton should not be affected significantly[23,26,27]. The α-(1 → 2) linkage can also be confirmed by the chemical shift of C-1 of the newly added sugar. Its chemical shifts for the α and β anomers are different only in the case of α-(1 → 2) linkage and appear at 97.5 and 99.0 ppm, respectively, and are easily distinguishable from other linkages[26]. These two peaks are also observed in the ^{13}C NMR spectrum of the product in Fig. 4B at 97.3 and 98.7 ppm, respectively.

DISCUSSION

Although C-6 is the expected position for the addition of new glucosyl residues, modifications made at position 6 have little apparent effect on the ability of methyl α-D-glucopyranoside analogues to serve as acceptors[13]. We have shown here that

methyl 6-bromo-α-D-glucopyranoside is a good acceptor, and the disaccharide thus synthesized has a α-(1 → 3) linkage. This result provides the first example of the formation of a 100% α-(1 → 3) linkage by dextransucrase from *S. sanguis*. Our observation that a small proportion (< 2%) of the product formed from methyl α-D-glucopyranoside had α-(1 → 3) linkages is in contrast to earlier findings[14,17] that only α-(1 → 6) bonds were formed with low molecular weight acceptors. However, Walker[17] has shown that branch α-(1 → 3) linkages may be formed in small proportions when isomaltose oligosaccharides with a chain length of 6 or higher are employed as acceptors. Robyt and Taniguchi[26] have shown that α-(1 → 3) bonds are introduced as branch linkages when enzyme linked dextranyl chains, formed by de novo polymerization, are transferred from the enzyme to an acceptor. Our results show that monosaccharides modified at position-6 are good acceptors at position-3. The appearance of α-(1 → 3) linked glucosyl residues in products formed from methyl α-D-glucopyranoside and from C-6 blocked analogues indicates that the single enzyme from *S. sanguis* can catalyze the formation of both types of linkages, but preferentially the α-(1 → 6), and that the bond represents a nonbranching linkage, since the new sugar is at the nonreducing terminus. The transfer of single glucosyl residues to acceptors indicates that the *S. sanguis* enzyme does not require the transfer of preformed dextranyl chains in order to generate α-(1 → 3) linkages.

It was surprising to see that in the reaction with methyl 6-bromo-6-deoxy-α-D-glucopyranoside as acceptor, the resulting trisaccharide had only α-(1 → 3) linkages. The nonreducing end of the disaccharide, formed by addition of the first glucose, has an unsubstituted C-6. If the trisaccharide is formed by utilizing the disaccharide as an acceptor, it may be expected that the transfer would take place at C-6 of the nonreducing end. However, the results show that transfer takes place at C-3 of the nonreducing end. This raises the question whether α-(1 → 3) linked acceptors yield only products with only α-(1 → 3) linkages. To address this question, nigerose, which is an α-(1 → 3) linked disaccharide of glucose, was employed as the acceptor in order to characterize the resulting products. As seen in Table I, nigerose gave multiple acceptor products. Significant amounts of low molecular weight products were not observed probably because the products themselves are good acceptors. The high molecular weight products were not well resolved by paper chromatography. So the mixture of products migrating between R_s 0.05–0.19 was eluted from the chromatogram. The ^{13}C NMR spectrum (not shown) of the mixture of products indicated that all new glucose units were added to the nonreducing ends to form α-(1 → 6) linkages. This suggests that with methyl 6-bromo-6-deoxy-α-D-glucopyranoside as acceptor, the trisaccharide product is synthesized, not by employing the intermediate disaccharide as acceptor, but by a single-chain mechanism, without the release of the intermediate disaccharide. This would imply that once an α-(1 → 3) bond is formed, the next glucose unit will be added in a single-chain fashion to form another α-(1 → 3) bond. Any of the intermediates that are released and later serve as acceptors add the new glucose

unit at position-6. When methyl 6-bromo-6-deoxy-α-D-glucopyranoside was employed as an acceptor, very little of the trisaccharide was produced[13]. Thus, formation of α-(1 → 3) links by the single-chain mechanism is not favored, and the product is released from the enzyme as a disaccharide. As an acceptor, the disaccharide cannot compete with the overwhelmingly large amount of monosaccharide, which is also a better acceptor. Even after complete reaction (Table I, line 6) the molar ratio of monosaccharide-to-disaccharide was 3.5 : 1. So the trisaccharide had no detectable α-(1 → 6) linkage.

We have previously[13] studied the acceptor activities of fifteen different analogues of methyl α-D-glucopyranoside modified at position-6. Based on these results it was proposed that when position-6 is blocked the acceptor binds to the enzyme in a mode represented by a 180° flip around the C-1–C-4 axis of the pyranose ring, which would result in the approximate exchange in space of the hydroxyl groups at C-3 and C-6, and thereby put C-3 adjacent to the active site. Our observation that an α-(1 → 3) linkage is formed when methyl 6-bromo-6-deoxy-α-D-glucopyranoside is used as an acceptor further supports this model. Flipping of the molecule can take place after it binds to the acceptor site. Such rotation at the active site has been shown for UDP-galactose-4-epimerase[29]. Since the acceptor specificity of dextransucrase is very broad, such rotation at the acceptor site may be sterically feasible. On the other hand, the flipping may take place before it enters the acceptor site. Since the enzyme usually makes α-(1 → 6) linkages, the acceptor molecules will usually enter the acceptor site in the unflipped orientation. Only when C-6 is blocked is there productive binding in the flipped orientation.

Acceptor activity as well as total activity were significantly decreased when C-6 of the nonreducing end of maltose was modified (Table I). However, enough of the acceptor product could be isolated and characterized. Why the next glucose is transfered to the C-2 position is not clear. This does not agree with the proposed model for monosaccharides. This probably arises from the best possible fit of the acceptor at the active site. More oligosaccharide acceptors need to be tested to fully understand the precise mechanism of the reaction. 6′-Bromo-6′-deoxymaltose appears to be similar to raffinose, lactose, and cellobiose in that the glucosyl group is transferred not to the nonreducing end but to the 2-position of the glucose residue in raffinose[30], and the 2-position of the reducing residue in lactose[31], cellobiose[32], and 6′-bromo-6′-deoxymaltose. Formation of an α-(1 → 2) linkage by dextransucrase from *S. sanguis* has not been reported before. However, dextransucrase from other oganisms have been shown to form α-(1 → 2) linkages with some acceptors[30–33]. Dextransucrase from *L. mesenteroides* B-1299 is also known to produce dextran containing α-(1 → 6), α-(1 → 3), and α-(1 → 2) linkages[34]. In conclusion, it can be said that dextransucrase from *S. sanguis* is capable of synthesizing α-(1 → 6), α-(1 → 3), and α-(1 → 2) linkages in an acceptor dependent reaction. Normally the α-(1 → 6) bond is formed. When C-6 is blocked, there is productive binding in other modes to give α-(1 → 3) and α-(1 → 2) linkages.

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