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Monosaccharide Acceptor Substrate Specificity of Dextransucrase[1]

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The acceptor substrate specificity of dextransucrase from *Streptococcus sanguis* 10558 was examined utilizing analogs of methyl-α-D-glucopyranoside. The analogs include a series of α-methyl glycosides of several epimers, derivatives blocked at position 6, 6 blocked epimers, several β-methyl derivatives, and L-sugars. The products formed during reactions between the analogs, the enzyme and radiolabeled sucrose were separated by paper chromatography. This analysis permitted the simultaneous evaluation of transfer as well as polymerization reaction. All analogs examined served as acceptors with varying degrees of effectiveness. From the data obtained, a rough model for interaction of the enzyme with acceptors is proposed. Structural modifications at C2 and C4 produced the most significant alteration in the ability to serve as acceptors, and also inhibited the total catalytic activity of the enzyme. The enzyme is capable of using sugars in either $^4C_1$ or $^1C_4$ conformations. In addition, sugars blocked at C6, the normal site of glucose addition, can serve as good acceptors and therefore probably bind to the enzyme in a different orientation. The broad spectrum of analogs that can serve as acceptor suggests that the acceptor site has some flexibility.

INTRODUCTION

Dextransucrase (EC 2.4.1.5) catalyzes the formation of dextran using sucrose as a glucosyl donor substrate (*1*). Dextran, a polysaccharide produced by the enzyme from *Streptococcus sanguis* 10558 is a polymer of glucose in which the bonding is principally α-(1 → 6) with a small proportion of α-(1 → 3) (*2, 3*). Polymer formation can proceed in either of two pathways. The first of these involves a sequential transfer of glucose unit of sucrose to the nonreducing positions of a growing chain or to an added acceptor substrate (*4–10*). This pathway is analogous to the pathway described for the formation of glycogen from UDP-glucose, and is referred to as glucosyl transfer. The second pathway proceeds independently of acceptors, and involves a mechanism whereby chain growth occurs at the reducing terminus (*11–13*). This pathway appears to be a true polymerization, since new chains must be initiated and extended.

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A variety of compounds have been shown to serve as acceptors in the glucosyl transfer reaction. These range from simple glycosides and oligosaccharides containing α-linked glucosyl residues to dextran itself (*4–10*). Some acceptors, such as fructose, react so that only a single product is generated (*5, 9*). Others, such as maltose, isomaltose, and methyl-α-D-glucopyranoside, yield a homologous series of oligosaccharides with an increasing number of newly added glucosyl residues (*5, 10*). Compounds in the latter group therefore undergo multiple catalytic cycles that result in the serial transfers. Members of the former group, on the other hand, do not appear to have this capacity.

In previous works (*14–16*) we have systematically evaluated structural requirements for donor substrates. These studies were based on the demonstration that α-fluoroglucopyranoside is a donor substrate with kinetic parameters comparable to sucrose (*14*) and allowed the examination of a series of epimers of fluoroglucose (*15*). In addition, analogs of sucrose that were halogenated at primary carbons were studied (*16*). We have continued these studies with a systematic investigation of the structural characteristics of acceptor substrates, using the simple glycoside, methyl-α-D-glucopyranoside, as a reference acceptor. Although this compound is not the most effective acceptor, its K_m (80 mM) makes it a reasonable reference. Its simple structure allows an examination of the structural variants that are either available commercially or can be synthesized by established procedures. The variants employed represent epimers and substitutions at individual positions in the molecule. A systematic study of this type has not been reported previously for this enzyme.

Earlier studies on the effectiveness of compounds as acceptor substrates for dextransucrase have utilized radiolabeled acceptors or labeled sucrose, and have measured the incorporation of isotope into products. Utilization of labeled acceptors gives direct evidence that products are derived from the acceptor. However, the availability of radiolabeled compounds of this type is limited, and the utilization of labeled sucrose is a reasonable alternative. Moreover, paper chromatographic analysis of reactions employing radioactive sucrose permits the simultaneous measurement of the transfer as well as the polymerization reaction. The analysis also provides the ability to determine the number of products and information on the extent of glucosylation based on their relative mobilities.

EXPERIMENTAL

Materials

Reagents used in this study were obtained from the following sources: methyl glycosides of α-D-glucopyranose, α-D-mannopyranose, α-D-galactopyranose, α-D-altropyranose, α-D-xylopyranose, 6-deoxy-α-D-glucopyranose, 6-deoxy-α-L-galactopyranose, β-D-glucopyranose, and 6-deoxy-β-D-glucopyranose were purchased from Sigma Chemical Co. (St. Louis, MO) along with invertase; Silica gel 60, plastic-backed TLC plates (Silica gel 60 and Silica gel 60F-254) were from E. Merck (Gibbstown, NJ); ion-exchange resin was from Bio-Rad laboratories

(Richmond, CA); carbon tetrabromide was from Aldrich Chemical Co. (Milwaukee, WI); β-D-allopyranose was from Pfanstiehl Laboratories (Waukeegan, IL); [*glucose*-^{14}C]sucrose was from New England Nuclear Corp. (Boston, MA); [^{14}C]methyl-α-D-glucopyranoside was from Amersham (Arlington Heights, IL). Other sugars were prepared according to procedures described under Methods.

Highly purified dextransucrase was obtained by the method of Grahame and Mayer (*17*). The purified enzyme was in an inactive state, since it was in a 0.1% SDS solution. Reactivation was accomplished by the addition of an equal volume of 2% Triton X-100. All other materials were of reagent grade and available commercially from common chemical sources.

Methods

Synthetic methods. Synthesis of 6-chloro and 6-bromo derivatives of the methyl-α-D-glycopyranosides was carried out according to the method of Anisuzzaman and Whistler (*18*). The specific sugar was reacted with the appropriate carbon tetrahalide in the presence of pyridine and triphenylphosphine. Following the reaction, the pyridine was removed by evaporation, and the sugars were dissolved in water, which left the majority of the other products as a precipitate (carbon tetrahalide, triphenyl phosphine and triphenyl phosphine oxide). These were removed by filtration and the sugar solutions were evaporated to dryness. The products were then purified by crystallization twice from an ethanol/hexane mixture. Since methyl-6-bromo-α-D-mannopyranoside could not be crystallized, it was first purified by chromatography of an aqueous solution on a column of AG1-X8, 20–50 mesh, hydroxide form, in order to remove halomethyl triphenylphosphonium halides, which are by-products of the reaction, and which are effective inhibitors of dextransucrase (*16*). Further purification was achieved by chromatography on a silica gel column. Methyl-6-iodo-α-D-glucopyranoside was obtained in a reaction of 1.0 g of methyl-6-bromo-α-D-glucopyranoside with 2.8 g KI in 10 ml dimethylformamide at 55°C for 16 h. The reaction mixture was filtered, the filtrate evaporated to dryness, and the sugar dissolved in $CHCl_3$. Following removal of the insoluble inorganic materials by filtration, the solution was evaporated to dryness and the product was crystallized from a mixture of ethanol and hexane. The yield was 0.8 g.

Methyl allosides were prepared from β-D-allose according to procedure of Evans and Angyal (*19*), in which β-D-allose was reacted with methyl orthoformate in the presence of $SrCl_2$ and acetyl chloride.

The synthesis of methyl-6-deoxy-D-galactosides and methyl-6-deoxy-L-mannosides were from 6-deoxy-D-galactose and 6-deoxy-L-mannose by glycosylation with 1% methanolic HCl, which was generated from acetyl chloride (*20, 21*).

All crystalline compounds were characterized by their melting points. Proton and carbon NMR spectra were obtained for all synthesized materials, and were found to be consistent with those reported in the literature (*18, 19, 22, 23*).

Chromatographic methods. The purification of synthesized sugars was accomplished by chromatography on silica gel columns (2 × 30 cm) using solvent system I consisting of ethyl acetate/ethanol/water (45/5/3). The progress of reaction and

the elution of compounds from chromatographic columns were monitored by thin layer chromatography on plastic-backed TLC sheets with or without fluorescent indicator, using solvent system I. After development, aromatic compounds were visualized with uv light, and sugars were detected by charring after spraying with 25% H_2SO_4 in methanol.

Paper chromatography was carried out on Whatman 1MM paper in a descending manner until the solvent front had moved 50 cm beyond the origin. Solvent system II was *n*-propanol/ethyl acetate/water (6/1/3), and solvent system III was butanone/acetic acid/boric acid-saturated water (9/1/1). Chromatograms were visualized by staining with silver nitrate–NaOH (*24*). Radioactive chromatograms were analyzed by cutting into 1-cm strips which were counted in 10 ml scintillation fluid (4.02 g PPO and 0.1 g POPOP per liter of spectral grade toluene) in a liquid scintillation counter.

Enzymatic methods. Dextransucrase was assayed by procedures described previously (*25*). Acceptor activity of the analogs of methyl-α-D-glucopyranoside were determined in reactions consisting of dextransucrase (0.05 units), [*glucose*-^{14}C]sucrose (20 mM, 3×10^5 dpm), 0.1 M phosphate buffer, pH 6.0, and the analog at concentrations specified in Table 1. Reactions were carried out in a 60-μl volume at 37°C for 4 h. Following reaction, 20-μl aliquots were spotted on Whatman 1MM paper and chromatographed in solvent system II and analyzed as described above. Radioactivity that comigrated with sucrose was eluted from strips with water, treated with 4 units of invertase at pH 4.5 to destroy residual sucrose, and then rechromatographed in solvent system III as described above.

RESULTS

The acceptor activities of the analogs of methyl-α-D-glucopyranoside were examined in a series of studies utilizing saturating concentrations of [*glucose*-^{14}C]sucrose as the labeled substrate in the presence of a large excess of the test acceptor. This was done in an effort to ensure that the acceptors would be present at saturating concentrations. Paper chromatographic analyses of the products formed from methyl-α-D-glucopyranoside (**2**) and methyl-6-deoxy α-D-glucopyranoside (**10**) are shown in Fig. 1. The figure illustrates the fact that both polymeric (nonmobile) and oligomeric products are generated in the reactions. In addition, it can be seen that methyl-α-D-glucopyranoside is an acceptor substrate that yields a series of oligosaccharide products, as reported previously (*5, 7, 9*), while methyl-6-deoxy-α-D-glucopyranoside generates a major product and trace amounts of other products. Radioactivity migrating slightly faster than sucrose at R_s 1.1 in Fig. 1b represents glucose produced due to hydrolysis of sucrose by dextransucrase (*26*). Similarly, analogs of methyl-α-D-glucopyranoside were investigated for their ability to serve as acceptors in the glucosyl transfer reaction catalyzed by the enzyme. The results of these investigations are summarized in Table 1. For each compound the concentration employed is shown along with the percentage of the total isotope that is attributed to the polymerization reaction (nonmobile). The table also provides a schematic representation of the paper chromatographic analysis of the

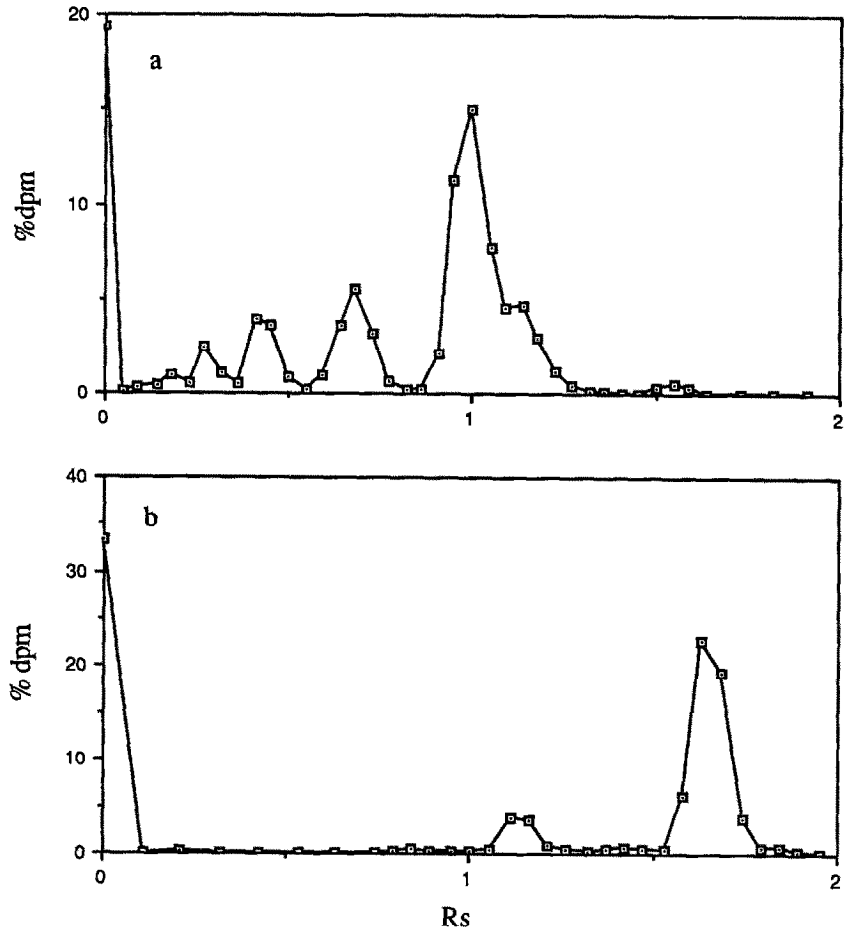

FIG. 1. Formation of acceptor products. Reactions were carried out as described under Methods using: (a) methyl-α-D-glucopyranoside (**2**) and (b) methyl-6-deoxy-α-D-glucopyranoside (**10**) as acceptors at 350 mM concentrations. After 4 h of reaction at 37°C, 20-μl aliquots were spotted on Whatman 1MM chromatography paper, and developed and analyzed as described under Methods. The data are presented as the percentage of the total dpm spotted on the chromatogram as a function of migration distance relative to sucrose (Rs).

oligomeric products formed by glucosyl transfer to the acceptors. These data are summarized with the percentage of the dpm that appear in the acceptor products, and the percentage of the total isotope in all reaction products, which is a representation of the total enzyme activity. In addition, the ratio of isotope in the acceptor products to the polymerization product is shown. Glucose produced by enzymatic hydrolysis of sucrose has not been counted as acceptor product.

Most of the compounds undergo multiple transfers that result in the distribution of the isotope in a series of oligosaccharides, as typified by methyl-α-D-glucopyranoside (**2**). Other compounds generate a single product, or one major product

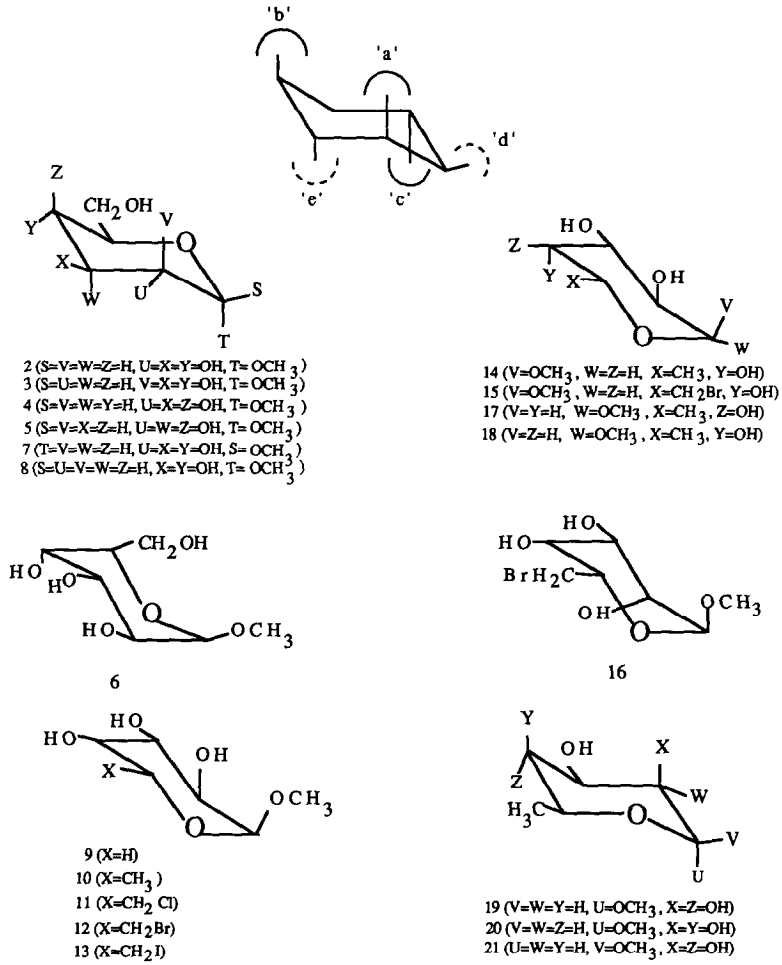

FIG. 2. Proposed model for binding of acceptors to dextransucrase. The binding modes of acceptors have been shown relative to that of methyl-α-D-glucopyranoside (**2**). Acceptor activity is the least when bulky groups are positioned at a, b, or c, intermediate at d or e, and not affected at other positions. Structures are schematically drawn in the most favored conformation in such a way as to maintain the positions of C1 and C4 fixed. Compounds which have C6 blocked have been rotated by 180° around the C1–C4 axis, thereby placing C3 at the position previously occupied by C5. The numbers designating the compounds refer to the order in which they appear in Table 1.

with trace amounts of secondary ones as in the case of methyl-6-deoxy-α-D-glucopyranoside (**10**). All the compounds examined act as acceptors to some degree. The poorest acceptors result in about 2 to 3% conversion from sucrose to acceptor products, while the best ones show about 60% yield. Based on acceptor activities shown in Table 1, a model has been developed to understand the steric requirements for being a good acceptor. According to this model, there is a strong steric hindrance when hydroxyl groups or other bulky groups are in positions a, b, and c, as shown in Fig. 2, resulting in poor acceptor activity. When these

bulky groups are in positions d and e there is mild steric hindrance resulting in intermediate acceptor activity. Modification in any other position does not affect the acceptor abilities of the compounds. For the purpose of this investigation the acceptor activities have been broadly classified based on the percentage dpm in the total acceptor product formed (Table 1) as low (<10%), intermediate (10–25%), and high (>25%).

The compounds examined fall into several groups based on the nature of the modification. The first group is a series of epimers of methyl-α-D-glucopyranoside in which the configuration of the hydroxyl groups at each ring position has been inverted. These are compounds **2** through **6** of Table 1. Epimerization of individual hydroxyl groups of methyl-α-D-glucopyranoside diminishes the ability of the sugars to serve as acceptors. Inversions at positions C2 (**3**) or C4 (**5**) have the most pronounced effects, and it would appear that these alterations are the least tolerated by the enzyme because of steric interference at positions a and b, respectively. In addition to being a poor acceptor the D-manno-epimer (**3**) is a significant inhibitor of the total enzyme activity as seen by the sharp reduction in total product formation. This is due not only to the inability of the compound to serve as an acceptor, but also to the fact that it is an inhibitor of polymerization. While methyl-α-D-galactopyranoside (**5**) also is an inhibitor, its effect is not as pronounced as that observed with the manno-derivative. The other epimer, methyl-α-D-allopyranoside (**4**), shows intermediate acceptor activity due to interaction at position e. Methyl-α-D-altropyranoside (**6**), which contains inversions at both C2 and C3, may be expected to be a very poor acceptor due to interaction with the enzyme at positions a and e. However, it is known that methyl-α-D-altropyranoside exists in solution as a 1 : 1 mixture of $^4C_1$ and $^1C_4$ conformations (*27*). In the $^1C_4$ conformation it has equatorially oriented hydroxyl groups at C2 and C3. The equatorial methoxyl group at C1 experiences a mild steric hindrance at position d resulting in intermediate acceptor activity. Due to similar interaction at C1, methyl-β-D-glucopyranoside (**7**) also shows intermediate acceptor activity. The elimination of the hydroxyl group at C2 (**8**) results in a compound that is equivalent to the parent compound (**2**) in terms of acceptor activity.

The epimeric series was augmented with a comparison of modifications at position 6 of D-gluco-, D-manno-, and D-galactopyranosides (**9–18**). These include the removal of C6 (**9**), the removal of oxygen at C6 (**10, 14, 17,** and **18**), and the substitution of hydroxyl groups with halogens at C6 (**11–13, 15, 16**). A comparison of the methyl-α-D-glucopyranoside analogs (**10–13** and **17**) indicates that these modifications had no effect on the ability to serve as an acceptor, nor on the total catalytic activity of the enzyme. Even substitution with bulky halogens, such as bromide or iodine atoms, were without significant impact. This was a surprising observation, since the 6 position is the expected position for the addition of new glucosyl residues (*6, 9*). Thus, in these cases new glucosyl residues are added at some other position. Since the enzyme is known to catalyze the formation of both α-(1 → 6) and α-(1 → 3) bonds it is possible that when position 6 is blocked new glucosyl residues are added at position 3. For this to happen the acceptor molecule should bind at the active site in a different orientation. Considering the broad specificity of the enzyme for acceptors it is likely that the enzyme can accomodate

TABLE 1

Acceptor Activities of Methyl α-D-Glucopyranoside Analogs

Acceptor	Concn. (mM)	Polymer formed (% dpm)	Products formed from acceptor (% dpm)										Total acceptor product (% dpm)	Total product (% dpm)	Acceptor/ polymer product ratio
			R_s:	0.4			0.8		1.2	1.6					
1 None	—	77.1											—	77.1	—
2 Methyl-α-D-glucopyranoside	350	19.4	0.4	1.4	3.9	8.8	14.2	37.2		0.9			66.8	86.2	3.44
3 Methyl-α-D-mannopyranoside	350	11.6				0.3	0.9	0.9	0.4				2.5	14.1	0.22
4 Methyl-α-D-galactopyranoside	350	40.7			0.8		1.8	0.9		2.7			6.2	46.9	0.15
5 Methyl-α-D-allopyranoside	350	62.6			5.1		3.0	13.7		1.0			22.8	85.4	0.36
6 Methyl-α-D-altropyranoside	350	61.4			3.0		2.5	3.7	1.3	2.5			13.0	74.4	0.21
7 Methyl-β-D-glucopyranoside	350	53.4			8.9			1.2			1.8		11.9	65.3	0.22
8 Methyl-2-deoxy-α-D-glucopyranoside	350	39.1		0.4	1.5	3.6		12.2[a]	43.0				60.9[a]	100.0[a]	1.53[a]
9 Methyl-α-D-xylopyranoside	350	52.4			0.9		1.8	3.5	22.0	1.6			29.8	82.2	0.57
10 Methyl-6-deoxy-α-D-glucopyranoside	350	33.3								1.4	52.9		54.3	87.6	1.63
11 Methyl-6-chloro-α-D-glucopyranoside	350	21.5								0.8	68.3		69.1	90.6	3.21
12 Methyl-6-bromo-α-D-glucopyranoside	350	13.7								1.3	70.8		72.1	85.8	5.26
13 Methyl-6-iodo-α-D-glucopyranoside	350	9.2									79.6		79.6	88.8	8.65

14	Methyl-6-deoxy-α-D-galactopyranoside	350	20.7				1.9		61.8		63.7	84.4	3.08
15	Methyl-6-bromo-α-D-galactopyranoside	200	29.6					0.3	38.5		38.8	68.4	1.31
16	Methyl-6-bromo-α-D-mannopyranoside	350	30.2	4.9							4.9	35.1	0.16
17	Methyl-6-deoxy-β-D-glucopyranoside	350	66.1						7.6		7.6	73.7	0.11
18	Methyl-6-deoxy-β-D-galactopyranoside	350	9.0	1.5			3.4		22.5		27.4	36.4	3.04
19	Methyl-6-deoxy-α-L-mannopyranoside	300	45.0	5.1	2.5		1.0		22.2		30.8	75.8	0.65
20	Methyl-6-deoxy-α-L-galactopyranoside	350	54.9	1.4			2.2	5.3			9.9	64.8	0.18
21	Methyl-6-deoxy-β-L-mannopyranoside	200	64.2	2.9	4.3		3.1		3.0	1.5	14.8	79.0	0.23
22	Methyl-6-deoxy-α-D-galactofuranoside	110	68.2	1.6	6.2	4.0	3.3		4.5		19.6	87.8	0.29
23	Methyl-6-deoxy-β-D-galactofuranoside	350	58.9	2.2	2.9		4.3		10.2		19.6	78.5	0.33
24	Methyl-β-D-allofuranoside	80	58.1	4.3	3.7		3.5				11.5	69.6	0.20

Note. Comparison of acceptor activities of analogs of methyl-α-D-glucopyranoside. Reactions were carried out as described under Methods using the analogs at the concentrations shown in the table. After 4 h aliquots were spotted on Whatman 1MM paper. The chromatograms were developed and analyzed for radioactivity as described under Methods. The percentage of total dpm on the chromatogram that was located at the origin was taken as a measure of the polymer formed. A schematic representation of the paper chromatographic analysis of the low molecular weight products along with the percentage dpm associated with each component is shown in the center column. R_s value represents the distance migrated relative to sucrose. The percentage of total dpm in the oligosaccharide peaks is presented in the next data column as the total acceptor products formed, while the sum of the acceptor products and the polymerized products is represented as the total product. The last column is the ratio of acceptor products to polymer formed.

[a] Invertase treatment was not performed. So the number also includes unreacted as well as hydrolyzed sucrose.

such variations in binding. This alternate mode of binding would be represented by a 180° flip around the C1–C4 axis of the pyranose ring, which would result in the approximate exchange in space of the hydroxyl groups at C3 and C6 and thereby put C3 adjacent to the active site. Binding in this flipped mode, the molecules do not encounter any of the steric interactions shown in Fig. 2. Thus these compounds are all good acceptors. Similarly, acceptor activities of methyl-α-D-mannopyranosides and methyl-α-D-galactopyranosides modified at position 6 (**14–16**) can also be explained if they bind to the active site in the flipped orientation of the $^4C_1$ conformation (Fig. 2). Thus, 6-deoxy and 6-bromo analogs of methyl-α-D-galactopyranoside (**14, 15**) are good acceptors. The corresponding β-methyl analog (**18**) shows intermediate activity due to interaction at position d. Methyl-6-deoxy-α-D-mannopyranoside (**16**) experiences steric interaction at position c resulting in low acceptor activity. Some 6-deoxy-L-isomers (**19–21**) were also examined. Their binding at the active site is shown (Fig. 2) in the flipped orientation of the more predominant $^1C_4$ conformation. Their acceptor activities are also in agreement with the proposed model. Structures of furonosides are significantly different from pyranosides. However, the furanosides examined (**22–24**) showed reasonable acceptor activities suggesting that the enzyme specificity is rather broad.

DISCUSSION

The present report focuses attention on the structural characteristics of compounds that serve as acceptors in the glucosyl transfer reaction. Using methyl-α-D-glucopyranoside as a reference, we have attempted to make a systematic examination of the effect of variations and substitutions of groups on the sugar ring. The results indicate that all the analogs have some capacity to serve as acceptor substrates. All of the compounds examined have some activity, including ones that have structural variance at C2 and C4 (D-manno and D-galacto) which introduce the least tolerated modifications. Inversion at C1 and C3 reduces the relative acceptor activity but not to the extent as inversions at C2 and C4. The diminished acceptor activities of these epimers could be the result of steric interference of the reoriented hydroxyl groups or the absence of positive hydrogen bonding to the enzyme by properly oriented hydroxyls. The fact that methyl-2-deoxy-α-D-glucopyranoside (**8**) acts as a good acceptor suggests that hydrogen bonding at C2 is not essential and that the steric model is more probable. Similar changes at other positions have not been studied.

The finding that a wide range of sugars, including L sugars, β-methyl anomers, and furanosides, serve as acceptors reflects the broad specificity that the enzyme has for acceptors. These observations suggest that the binding site for acceptors is sufficiently flexible to tolerate the broad range of structural variations. The fact that some of the derivatives are superior to others suggests differences in ability to bind to the enzyme, and that some of the binding interactions are favored and more important.

Using the reaction with methyl-α-D-glucopyranoside (**2**) as reference, the major-

ity of compounds examined appear to inhibit the overall rate of reaction. This can be seen by comparison of the sums of the oligomeric and polymeric products. It is of interest to note that the only compounds that do not inhibit significantly are analogs of methyl-α-D-glucopyranoside that are modified at position 6 (**9–13**). These modifications also had little apparent effect on the ability of the analogs to serve as acceptors. Another interesting characteristic of analogs modified at position 6 is that they essentially yield a single reaction product. All others produce homologous series of products. This is probably because the major products in these cases do not serve as good acceptors in multiple catalytic cycles.

REFERENCES

1. HEHRE, E. J. (1941) *Science* **93,** 237–238.
2. SIDEBOTHAM, R. L. (1974) *Adv. Carbohydr. Chem. Biochem.* **30,** 371–444.
3. ARNETT, A. T., AND MAYER, R. M. (1975) *Carbohydr. Res.* **42,** 339–345.
4. HEHRE, E. J. (1968) *J. Polymer Sci.* **23,** 239–244.
5. KOEPSELL, H. J., TSUCHIYA, H. M., HELLMAN, N. N., KAZENKO, A., HOFFMAN, C. A., SHARPE, E. S., AND JACKSON, R. W. (1953) *J. Biol. Chem.* **200,** 793–801.
6. WALKER, G. J. (1973) *Carbohydr. Res.* **30,** 1–10.
7. MAYER, R. M., MATTHEWS, M. M., FUTERMAN, C. L., PARNAIK, V. K., AND JUNG, S. M. (1981) *Arch. Biochem. Biophys.* **208,** 278–287.
8. WALKER, G. J. (1980) *Carbohydr. Res.* **82,** 404–410.
9. ROBYT, J. R., AND EKLUND, S. H. (1983) *Carbohydr. Res.* **121,** 279–286.
10. MCCABE, M. M., AND SMITH, E. E. (1978) *Carbohydr. Res.* **63,** 223–239.
11. ROBYT, J. F., KIMBLE, B. K., AND WALSETH, T. F. (1974) *Arch. Biochem. Biophys.* **165,** 634–640.
12. LUZIO, G. A., PARNAIK, V. K., AND MAYER, R. M. (1983) *Carbohydr. Res.* **121,** 269–278.
13. DITSON, S. L., AND MAYER, R. M. (1984) *Carbohydr. Res.* **126,** 170–175.
14. JUNG, S. M., AND MAYER, R. M. (1981) *Arch. Biochem. Biophys.* **208,** 288–295.
15. GRIER, T. J., AND MAYER, R. M. (1981) *Arch. Biochem. Biophys.* **212,** 651–659.
16. BHATTACHARJEE, M. K., AND MAYER, R. M. (1985) *Carbohydr. Res.* **142,** 277–284.
17. GRAHAME, D. A., AND MAYER, R. M. (1985) *Carbohydr. Res.* **141,** 285–298.
18. ANISUZZAMAN, A. K., AND WHISTLER, R. L. (1978) *Carbohydr. Res.* **61,** 511–518.
19. EVANS, M. E., AND ANGYAL, S. J. (1972) *Carbohydr. Res.* **25,** 43–48.
20. CONCHIE, J., LEVVY, G. A., AND MARSH, C. A. (1957) *Adv. Carbohydr. Chem.* **12,** 157–187.
21. FEISER, L. F., AND FEISER, M. (1967) Reagents for Organic Synthesis, Vol. 1, pp. 11, Wiley, New York.
22. SEO, S., TOMITA, Y., TORI, K., AND YOSHIMURA, Y. (1978) *J. Amer. Chem. Soc.* **100,** 3331–3339.
23. BOCK, K., AND PEDERSON, C. (1983) *Adv. Carbohydr. Chem. Biochem.* **41,** 27–66.
24. TREVELYAN, W. E., PROCTOR, D. P., AND HARRISON, J. S. (1950) *Nature (London)* **166,** 444–445.
25. LUZIO, G. A., GRAHAME, D. A., AND MAYER, R. M. (1982) *Arch. Biochem. Biophys.* **216,** 751–757.
26. LUZIO, G. A., AND MAYER, R. M. (1983) *Carbohydr. Res.* **111,** 311–318.
27. STODDART, J. F. (1971) Stereochemistry of Carbohydrates, pp. 89, Wiley–Interscience, New York.