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ACTION OF α-1,6-GLUCAN GLUCOHYDROLASE ON OLIGOSACCHARIDES DERIVED FROM DEXTRAN

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ABSTRACT

The action of α-1,6-glucan glucohydrolase on α-(1→6)-D-glucosidic linkages in oligosaccharides that also contain an α-(1→2)-, α-(1→3)-, or α-(1→4)-D-glucosidic linkage has been investigated. The enzyme could hydrolyse α-(1→6)-D-glucosidic linkages from the non-reducing end, including those adjacent to an anomalous linkage. α-(1→6)-D-Glucosidic linkages at branch points were not hydrolysed, and the enzyme could neither hydrolyse nor by-pass the anomalous linkages. These properties of α-1,6-glucan glucohydrolase explain the limited hydrolysis of dextrans by the exo-enzyme. Hydrolysis of the main chain of α-(1→6)-D-glucans will always stop one D-glucose residue away from a branch point. The extent of hydrolysis by α-1,6-glucan glucohydrolase of some oligosaccharide products of the action on dextran of *Penicillium funiculosum* and *P. lilacinum* dextranase, respectively, has been compared. Differences in the specificity of the two endo-dextranases were revealed. The *Penicillium* enzymes may hydrolyse dextran B-512 to produce branched oligosaccharides that retain the same 1-unit and 2-unit side-chains that occur in dextran.

INTRODUCTION

α-1,6-Glucan glucohydrolase is an exo-dextranase that is present in several strains of *Streptococcus mitis*[1], and in other species of Streptococcus isolated from human dental plaque[2]. The enzyme completely hydrolyses isomaltose saccharides, by removing D-glucose residues one at a time from the non-reducing end[3]. A comparison of K_m values for isomaltose, isomaltopentaose, and dextran B-512 showed that the enzyme had equal affinity for these substrates despite the difference in their chain lengths. The action of the enzyme on dextrans was incomplete, the extent of hydrolysis being related to the proportion of α-(1→6)-D-glucosidic linkages. The limit of hydrolysis of dextran B-512 was 23%, and the molecular weight of the limit dextran was of the same order of magnitude as that of the original, native dextran. Since the limit dextran contained all the original α-(1→3)-D-glucosidic linkages, it seemed probable that the 5% of anomalous linkages present in dextran B-512 were responsible for arresting the action of the enzyme.

The present paper describes the action of α-1,6-glucan glucohydrolase on a number of oligosaccharides that contain the same linkages that occur in dextrans.

MATERIALS AND METHODS

Carbohydrates. — Maltotriose, *O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranose (**1**), *O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→6)-α-D-glucopyranose (**2**), *O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranose (**4**), *O*-α-D-glucopyranosyl-(1→4)-*O*-[α-D-glucopyranosyl-(1→6)]-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose (**5**), and *O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranose (**6**) were prepared by methods previously described[1]. 4,6-Di-*O*-(α-D-glucopyranosyl)-α-D-glucopyranose[4] (**3**) was a gift from Dr. I. J. Goldstein.

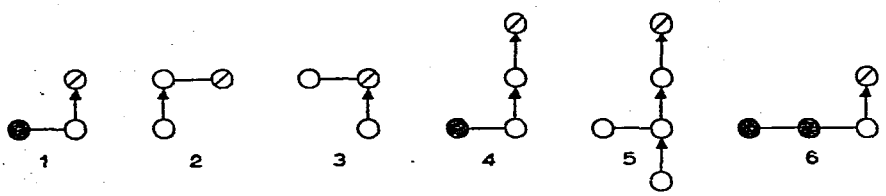


Fig. 1. Structures of oligosaccharides that contain α-(1→6)-D-glucosidic linkages (—) and α-(1→4)-D-glucosidic linkages (↑). ○, D-glucose residue; ⊘, reducing D-glucose residue; ●, D-glucose residue that is released by α-1,6-glucan glucohydrolase. The full names of the oligosaccharides are given in the Materials and Methods section.

Nigerotriose[5] was kindly provided by Dr. I. R. Johnston, and the hendecaacetate of *O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→3)-α-D-glucopyranose[6] (**7**) by Dr. K. Matsuda. *O*-α-D-Glucopyranosyl-(1→6)-*O*-[α-D-glucopyranosyl-(1→3)]-*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→6)-D-glucopyranose (**8**) was isolated from the reaction products of *Penicillium lilacinum* dextranase on dextran B-512 (F). *O*-α-D-Glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→6)-α-D-glucopyranose (**9**) was isolated by paper chromatography from the products of the action of *Penicillium funiculosum* dextranase on dextran B-512 (F).

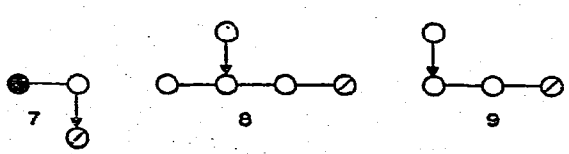


Fig. 2. Structures of oligosaccharides that contain α-(1→6)-D-glucosidic linkages (—) and α-(1→3)-D-glucosidic linkages (↓). Other details as in Fig. 1.

The trisaccharide 2,6-di-*O*-(α-D-glucopyranosyl)-α-D-glucopyranose[7], which was isolated from the acetolysis products of dextran NRRL B-1397, was a generous gift from Dr. M. Torii.

Dextran from *Leuconostoc mesenteroides* NCIB 2706 was prepared as described by Rees *et al.*[8]. A commercial preparation of dextran (mol. wt. 40,000) from *L. mesenteroides* NRRL B-512 (F) was supplied by the Pharmachem Corporation. Dextrans produced by *L. mesenteroides* NRRL B-512(F) and by *L. dextranicum* NRRL B-1375 (Birmingham strain) were kindly provided by Dr. A. Jeanes. These dextrans are described below as dextran B-512(F) and Birmingham dextran, respectively. The preparation of *Streptococcus bovis* NCDO 1253 dextran, and the sources of *L. mesenteroides* NRRL B-512 and B-1415 dextrans were described previously[3].

Total carbohydrate was estimated with a cysteine–sulphuric acid reagent[9]. D-Glucose, which was assayed with D-glucose oxidase reagent[10], could be accurately determined in the presence of oligosaccharides. Separations of branched oligosaccharides, and of mono- from di- and tri-saccharides were made by chromatography on Whatman No. 3MM paper in ethyl acetate–pyridine–water (10:4:3). Reducing power of branched oligosaccharides was determined with the Nelson reagent[11]. Reduction of periodate during the oxidation of branched oligosaccharides was determined by measuring the fall in absorbance at 223 nm.

Enzymes. — α-1,6-Glucan glucohydrolase was prepared by the method of Walker and Pulkownik[3]. Dextranase from *Penicillium funiculosum* QM474 (EC 3.2.11) was kindly provided by Dr. E. T. Reese. Dextranase from *Penicillium lilacinum* NRRL 896 was prepared by the method[2] previously described for *P. funiculosum* dextranase.

Units of all enzyme activities are defined as the amount which will release 1 μmole of product per min.

RESULTS

Action of α-1,6-glucan glucohydrolase on oligosaccharides containing the same linkages as dextran. — Each oligosaccharide (0.6 μmole) was incubated at 35° with enzyme (0.25 unit) in a digest (0.9 ml) that contained 25mM sodium citrate buffer (pH 6.0). Portions were withdrawn after 6 h, 24 h, and 3 days, for the determination of D-glucose. The other products, identified by paper chromatography, were those expected from the known structures of the model subtrates, which are shown diagrammatically in Figs. 1 and 2 where D-glucose residues that can be released by the enzyme are represented by ●. Saccharides **1** and **6** were completely hydrolysed to give D-glucose and maltose, while **4** gave D-glucose and maltotriose. Maltose and maltotriose were not degraded by the enzyme. Saccharide **7** was completely hydrolysed to D-glucose and nigerose, but **8** and **9** were not attacked. Nigerose and nigerotriose were not substrates for the enzyme. Kojibiose[3] was previously shown to be resistant to α-1,6-glucan glucohydrolase; neither the α-(1→2) nor the α-(1→6) linkage in 2,6-di-D-glucosyl-D-glucose was hydrolysed.

The relative rate of hydrolysis of several trisaccharide substrates was compared with that of isomaltotriose (Table I). The digests were sampled frequently during the early stages of the hydrolyses when the reaction rates were linear. Replacement of the linkage at the reducing end of isomaltotriose with an α-(1→3)- or an α-(1→4)-D-glucosidic linkage, or even with a sucrose linkage, did not markedly affect the rate of hydrolysis of the α-(1→6) linkage at the non-reducing end.

TABLE I

RELATIVE ACTIVITY OF α-*1,6*-GLUCAN GLUCOHYDROLASE ON THE α-(1→6)-D-GLUCOSIDIC LINKAGE AT THE NON-REDUCING END OF TRISACCHARIDES[a]

Trisaccharide	*Relative rate of hydrolysis*
Isomaltotriose	100
6^2-α-D-glucosylmaltose	160
6^2-α-D-glucosylnigerose	130
6^G-α-D-glucosylsucrose	75

[a]The digests (0.25 ml) contained trisaccharide (0.6 μmole), 25mM citrate buffer (pH 6.0), and α-1,6-glucan glucohydrolase (0.01 unit).

Properties of branched oligosaccharides prepared from dextrans by enzymic degradation. — Several α-(1→6)-D-glucosidic linkages in the vicinity of the branch points in *Leuconostoc dextranicum* (Birmingham) dextran were reported[12] to be resistant to hydrolysis by the extracellular endo-dextranases of *Penicillium sp.* Structural examination of the products of *P. lilacinum* and *P. funiculosum* dextranases on Birmingham dextran had suggested that the branched penta- and hexa-saccharides (B_5 and B_6) produced by the two enzymes were identical. They were reported to be mainly 3^3-α-D-glucosylisomaltosaccharides, but the possibility that B_6 might also contain 3^4-α-D-glucosylisomaltopentaose was not excluded. The branched oligosaccharides B_7 and B_8 produced by *P. lilacinum* dextranase were 3^4-α-D-glucosylisomaltosaccharides, possibly in admixture with isomers in which the branch point occurred closer to the non-reducing end. The structures of B_7 and B_8 produced from the same dextran by *P. funiculosum* dextranase were not examined.

Similar series of branched oligosaccharides have now been prepared from *Streptococcus bovis* NCDO 1253 dextran, from *L. mesenteroides* B-512(F) dextran, and from Pharmachem dextran, by the action of *P. funiculosum* dextranase. Another series was prepared from dextran B-512(F) with *P. lilacinum* dextranase. Each dextran (0.5 g) was incubated at 35° with dextranase (12 i.u.) in a digest (6 ml) containing 25mM sodium citrate buffer (pH 6.0). Portions were removed at intervals for determination of the reducing power, and when the reaction was complete, the digest was boiled to inactivate the enzyme. Each solution was deionized with Biodeminrolit (The Permutit Co.), and concentrated to a syrup. The products were separated by paper chromatography; rechromatography of the higher oligosaccharides B_6, B_7, and B_8 was required to achieve complete separation. The distance

moved by each branched oligosaccharide was greater than for the corresponding isomaltose oligosaccharide because of the increased mobility conferred by the introduction of any α-D-glucosidic linkage other than 1,6. A plot relating mobility to d.p. of the branched oligosaccharides was parallel to a similar plot for isomaltose oligosaccharides (Fig. 3), indicating that the two homologous series differed by a constant factor.

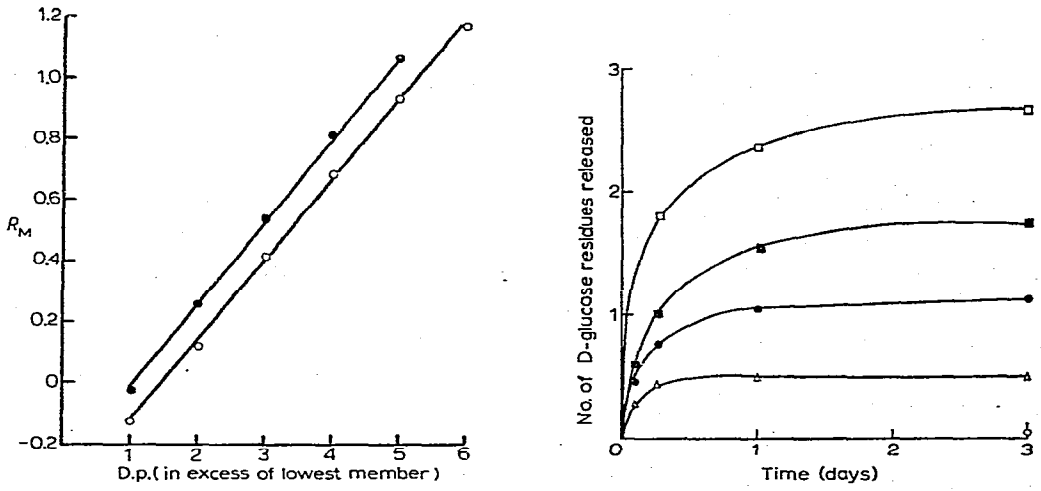


Fig. 3. R_M values (*i.e.*, $\log[(1/R_A - 1)]$ of isomaltose oligosaccharides (○), and branched oligosaccharides (●) that are end-products of *P. funiculosum* dextranase action on *S. bovis* dextran. R_A is (distance moved by oligosaccharide)/(distance moved by the lowest member of the series). The lowest members taken for the two series were isomaltose and branched tetrasaccharide, respectively.

Fig. 4. Action of α-1,6-glucan glucohydrolase on branched oligosaccharides derived from dextran B-512(F) (Pharmachem). ○, B_4; △, B_5; ●, B_6; ■, B_7; and □, B_8.

Confirmation that this factor was an α-(1→3)-D-glucosidic linkage was obtained by oxidation with sodium metaperiodate. Each branched oligosaccharide derived from *S. bovis* dextran was incubated with a 3-fold excess of 10mM periodate for 24 h in the dark at room temperature. Reduction of periodate was close to the theoretical values for 3-*O*-α-D-glucosylisomaltose saccharides (Table II). After completion of the oxidations, excess periodate was reduced with ethylene glycol, and the solutions were deionized with Biodeminrolit. Undegraded D-glucose residues remaining within the oligoaldehydes were determined with the Dische reagent[9]; portions were also hydrolysed for 2 h at 100° in 0.75M H_2SO_4, and D-glucose in the product was estimated with D-glucose oxidase reagent[10]. The results (Table II) broadly confirm that each oxidized, branched oligosaccharide contained one unchanged D-glucose residue. By contrast, after all the D-glucose residues in isomaltotetraose (IM_4) had been oxidized with periodate, no reaction was obtained with cysteine–H_2SO_4; nor, after hydrolysis, could D-glucose be determined with D-glucose oxidase.

TABLE II

PERIODATE OXIDATION OF BRANCHED OLIGOSACCHARIDES PRODUCED FROM *Streptococcus bovis* DEXTRAN

D.p.	*Periodate reduction (Mol.)*		*Unoxidized D-glucose (residues/molecule)*
	Calc.	*Found*	
4	7	6.6	0.8
5	9	8.8	1.0
6	11	10.9	0.9
7	13	11.8	1.2
IM_4	9	9.3	0

The branched oligosaccharides (0.6 μmole) derived from three dextrans by the action of *P. funiculosum* dextranase, and those derived from dextran B-512(F) by the action of *P. lilacinum* dextranase, were each incubated with α-1,6-glucan glucohydrolase (0.07 unit) for 24 h at pH 6.0 and 35°. The number of D-glucose residues released from the oligosaccharide products of *P. lilacinum* action were: B_5, 0; B_6, 0.5; B_7, 1.0. The results for the action of *S. mitis* α-1,6-glucan glucohydrolase on the oligosaccharides derived from *S. bovis* dextran and from Pharmachem dextran by the action of *P. funiculosum* dextranase are shown in Table III and Fig. 4, respectively. Since these results are different from those for the oligosaccharides prepared with *P. lilacinum* dextranase, it follows that the structure of the oligosaccharides series and also the action pattern of the two *Penicillium* dextranases must be different.

TABLE III

ACTION OF α-1,6-GLUCAN GLUCOHYDROLASE ON BRANCHED OLIGOSACCHARIDES FROM *S. bovis* DEXTRAN

Time (h)	*Number of D-glucose residues released*			
	B_4	B_5	B_6	B_7
0.75	0.04	0.16	0.68	0.66
1.5	0.04	0.23	0.80	0.98
3	—	0.25	0.95	1.30
24	0.04	0.27	1.03	1.80

The nature of the products that remained after completion of the enzyme reactions with the oligosaccharides prepared with *P. funiculosum* dextranase was examined by paper chromatography. They were those expected from the number of D-glucose residues released from each structure. Thus, there were no products from B_4, and the B_5 digests contained B_4, D-glucose, and unhydrolysed B_5 (resistant B_5). The products in the B_6 digests were B_5 (resistant B_5) and D-glucose.

Resistant B_5 from the two B_5 digests was eluted from the paper chromatograms with water, and subjected to a second incubation with enzyme, under the same conditions. There was no further release of D-glucose. Methylation analysis of the resistant pentasaccharide fraction from dextran B-512(F) yielded 2,3,4,6-tetra-*O*-methyl-D-glucose (1.97 mol.), 2,3,4-tri-*O*-methyl-D-glucose (1.95 mol.), and 2,4-di-*O*-methyl-D-glucose (1.00 mol.).

Branched pentasaccharides were also prepared with *P. funiculosum* dextranase, by the method described above, from *Leuconostoc dextranicum* NRRL B-1375 (Birmingham strain) dextran, from *L. mesenteroides* NCIB 2706 dextran, and from *L. mesenteroides* NRRL B-1415 dextran. Again, the number of D-glucose residues released from each B_5 was less than one (Table IV), indicating the presence of two isomers, one of which was resistant (rB_5), and the other susceptible (sB_5), to the action of α-1,6-glucan glucohydrolase.

TABLE IV

ACTION OF α-1,6-GLUCAN GLUCOHYDROLASE ON BRANCHED PENTASACCHARIDE FRACTIONS (B_5) OBTAINED FROM VARIOUS DEXTRANS

Source of dextran	D-*Glucose residues released from* B_5
S. bovis NCDO 1253	0.27
L. mesenteroides NRRL B-512(F)	0.32
Pharmachem dextran	0.50
L. dextranicum NRRL B-1375	0.41
L. mesenteroides NCIB 2706	0.40
L. mesenteroides NRRL B-1415	0.55

Action of α-1,6-glucan glucohydrolase on the side chains of dextran B-512. — Recent studies[13] on the length of the side chains of dextran B-512 revealed that 40% consisted of a single D-glucosyl residue, and that at least 45% contained two D-glucose residues. It was of interest to determine whether α-1,6-glucan glucohydrolase could remove a D-glucose residue from the 2-unit side-chains, thereby producing a limit dextran in which up to 85% of the side chains consisted of single D-glucosyl stubs. Attenuation of the side chains of dextran could be disclosed by the altered distribution of products obtained on hydrolysis with endo-dextranase. In particular, the proportion of the tetrasaccharide (B_4) among the branched products should increase substantially.

Limit dextran was prepared from dextran B-512 (33 mg) by the action of α-1,6-glucan glucohydrolase, by the method described previously[3]. When the reaction was complete, the conversion into D-glucose was 22%. The digest was boiled, and D-glucose was removed by dialysis against four changes of distilled water. The limit-dextran solution was then incubated with *P. funiculosum* endo-dextranase (5 units) in a total volume of 1.75 ml at pH 6.0 and 35° for 3 days. Untreated dextran was also hydrolysed with the endo-dextranase under the same conditions. The products were

fractionated by paper chromatography, the sugars were eluted into volumetric flasks, and the weight of each sugar was determined. The weight of branched oligosaccharides obtained from dextran amounted to 21.7% of the total products; the corresponding figure for limit dextran was 27.4%. The tetrasaccharide B_4 accounted for 27.5% of the weight of branched oligosaccharides obtained from dextran. Since the same proportion of B_4 (27.3%) was found in the branched products from limit dextran, no change in the length of the short side-chains of dextran after incubation with α-1,6-glucan glucohydrolase was indicated. These results are summarized in Table V.

TABLE V

PRODUCTS[a] OF *P. funiculosum* ENDO-DEXTRANASE ACTION ON DEXTRAN B-512 AND ITS LIMIT DEXTRAN

Product	*Dextran*	*Limit dextran*
Linear products[b]	20.90	13.81
Tetrasaccharide B_4	1.59	1.42
Branched oligosaccharides[c]	5.79	5.20
Total products	26.69	19.01

[a]Weight of the products is given in mg. [b]These comprise D-glucose, isomaltose, and a trace of isomaltotriose. [c]These comprise B_4–B_8 and traces of higher products.

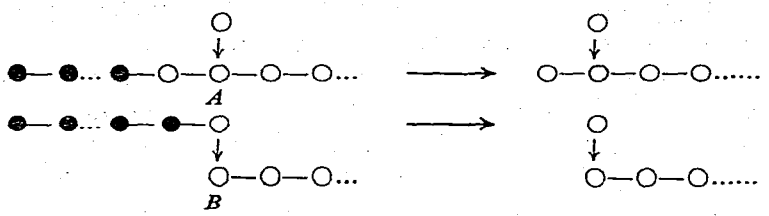
The action of α-1,6-glucan glucohydrolase on the branched-pentasaccharide fractions from dextran and limit dextran was examined. The extent of hydrolysis to D-glucose was identical (0.52 D-glucose residue released) for both pentasaccharides, showing that the proportion of rB_5 to sB_5 had remained the same.

DISCUSSION

Dextran is a collective name for a number of bacterial polysaccharides in which the chain-forming linkage is α-(1→6). In addition, almost all dextrans possess other α-D-glucosidic linkages which may occur either in the main chain, or at branch points, where they join side chains to the main chain. It has been suggested[3] that these anomalous linkages are responsible for the incomplete hydrolysis of dextrans by α-1,6-glucan glucohydrolase. A study of the enzymic hydrolysis of oligosaccharides that contain the same linkages as dextrans (Figs. 1 and 2) has now shown that this supposition was correct.

α-1,6-Glucan glucohydrolase had no action on α-(1→6) linkages attached to D-glucose residues that were also substituted at C-2, C-3, or C-4, and the enzyme could neither hydrolyse nor by-pass any anomalous linkage. α-(1→6)-D-Glucosidic linkages at the non-reducing end of oligosaccharides could be hydrolysed even when anomalous linkages were adjacent, provided a branch point was not involved. The nature of the adjacent, anomalous linkage had little effect on the rate of hydrolysis of the α-(1→6) linkage (Table I).

From these results, it can be expected that α-1,6-glucan glucohydrolase will act on dextran containing an anomalous linkage at a branch point (*A*) or in the main chain (*B*) to give the structures shown below.



Series of branched oligosaccharides prepared by the action of *P. funiculosum* and *P. lilacinum* dextranases on dextrans have also been tested as substrates for α-1,6-glucan glucohydrolase. It was assumed that their structures would be the same as those assigned to the oligosaccharides prepared by the same enzymes from Birmingham dextran, because the anomalous linkages in all the dextrans were α-(1→3)-D-glucosidic linkages which occurred at branch points. *S. bovis* dextran[3,14] and dextran B-512(F) contained fewer (~5%) branch linkages than Birmingham dextran (16%). This should not have affected the structures of the lower members, but multiple branching would be less likely to occur in the higher members of the series.

Branched oligosaccharide products of P. lilacinum *dextranase.* The fact that α-1,6-glucan glucohydrolase had no action on the branched pentasaccharide **8** formed part of the evidence that established the specificity of the exo-dextranase. The enzyme could not hydrolyse α-(1→6) linkages attached to a D-glucose residue at a branch point. The release of D-glucose from the branched hexasaccharide (B_6) indicated the presence of a component having a susceptible linkage at the non-reducing end, as in 3^3-α-D-glucosylisomaltopentaose, one of the structures[12,15] proposed for B_6. The second, possible structure for B_6, 3^4-α-D-glucosylisomaltopentaose, would be resistant to the action of the enzyme. The presence of both these isomers in the B_6 fraction would explain why less than 1 D-glucose residue was released by the glucohydrolase. After the reaction was complete, the pentasaccharide product was separated from D-glucose and unchanged substrate by paper chromatography, and subjected to methylation analysis. The results were those expected for a resistant branched pentasaccharide [2,3,4,6-tetra-*O*-methyl-D-glucose (2.0 mol.), 2,3,4-tri-*O*-methyl-D-glucose (2.0 mol.), and 2,4-di-*O*-methyl-D-glucose (1.0 mol.)].

The number of D-glucose residues released by α-1,6-glucan glucohydrolase from B_7 (1.0) was also in agreement with the structure, 3^4-α-D-glucosylisomaltohexaose, established[12] for this oligosaccharide.

Branched oligosaccharide products of P. funiculosum *dextranase.* The extent of hydrolysis of each member of the homologous series by α-1,6-glucan glucohydrolase was the same whether the oligosaccharides were derived from *S. bovis* or from dextran B-512(F) (*cf.* Table III and Fig. 4). Thus, the oligosaccharides, and consequently the dextrans from which they were prepared, were branched in a similar

manner. α-1,6-Glucan glucohydrolase released more D-glucose from each oligosaccharide than from the corresponding oligosaccharide prepared with *P. lilacinum* dextranase. The number of D-glucose residues released from B_6, B_7, and B_8 (1.0, 1.8, and 2.5, respectively) was close to that expected from 3^3-α-D-glucosylisomaltosaccharides. The structure of the branched hexasaccharide obtained with both *Penicillium* endo-dextranases was reported to be 3^3-α-D-glucosylisomaltopentaose, in possible admixture with 3^4-α-D-glucosylisomaltopentaose. The result of enzymic hydrolysis of B_6 prepared with *P. funiculosum* dextranase in the present work, however, indicated the absence of 3^4-α-D-glucosylisomaltopentaose, for the hexasaccharide was entirely converted into D-glucose and a branched pentasaccharide that was resistant to further hydrolysis. The results with B_7 and B_8 also indicated that these oligosaccharides could only contain minor amounts of 3^4-α-D-glucosylisomaltosaccharides. Thus, there are greater differences between the specificity of *P. lilacinum* dextranase and *P. funiculosum* dextranase than have hitherto been proposed.

Branched oligosaccharides arise from regions of dextran that are in the vicinity of branch points. Since it was established recently that dextran B-512 contains equal numbers of 1-unit and 2-unit side-chains[13], it follows that hydrolysis of this dextran with endo-dextranase must yield oligosaccharide products bearing 1-unit and 2-unit side-chains.

There is no possibility that *P. funiculosum* dextranase could decrease the length of the side chains from two to one D-glucosyl residue during any stage of the hydrolysis of dextran. The enzyme does not hydrolyse isomaltose, nor can it release a D-glucose residue from the non-reducing end of isomaltotriose or any other isomaltose saccharide[15]. The 2-unit side-chains of dextran B-512 must therefore appear among the products of endo-dextranase action.

The branched pentasaccharide product of *P. funiculosum* dextranase action on Birmingham dextran was shown[16] to be 3^3-α-D-glucosylisomaltotetraose (Fig. 5A), an oligosaccharide that would be resistant to α-1,6-glucan glucohydrolase. The results of methylation analysis were consistent with the fact that rB_5 from dextran

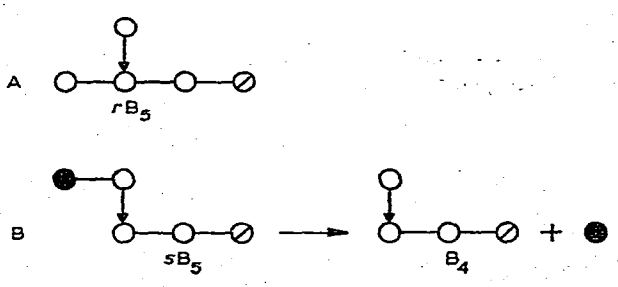


Fig. 5. A. Structure proposed for the branched pentasaccharide derived from Birmingham dextran. rB_5 and sB_5, Structures for B_5 that are resistant and susceptible, respectively, to the action of α-1,6-glucan glucohydrolase. B. Hydrolysis of sB_5 by α-1,6-glucan glucohydrolase to give B_4 and D-glucose (●).

B-512(F) was also 3^3-α-D-glucosylisomaltotetraose. Any structure proposed for sB_5 must satisfy the following requirements. (*1*) It must be consistent with the specificity of *P. funiculosum* dextranase action on dextran. The α-(1→6) linkage at the non-reducing side of the branch point may be hydrolysed, but two α-(1→6) linkages on the reducing side of the branch point are resistant[16]. (*2*) It must be consistent with the specificity of *S. mitis* α-1,6-glucan glucohydrolase. This enzyme can hydrolyse α-(1→6)-D-glucosidic linkages adjacent to an anomalous linkage only if no branch point is involved. (*3*) The structure must yield one D-glucose residue and 3^3-α-D-glucosylisomaltotriose on hydrolysis with α-1,6-glucan glucohydrolase.

3^3-α-Isomaltosylisomaltotriose is the only oligosaccharide that meets these requirements. We therefore propose that the pentasaccharide fraction obtained from *S. bovis* and dextrans B-512(F) with *P. funiculosum* dextranase contains two oligosaccharides, 3^3-α-isomaltosylisomaltotriose and 3^3-α-D-glucosylisomaltotetraose.

Further evidence for the structure of sB_5 was obtained by comparing B_4 obtained on hydrolysis of sB_5 (Fig. 5B) with the B_4 obtained on hydrolysis of dextran B-512(F) with *P. funiculosum* dextranase. Both tetrasaccharides were hydrolysed to D-glucose and isomaltotriose by *Cladosporium resinae* α-(1→3)-D-glucosidase. Since this enzyme cannot hydrolyse α-(1→3)-D-glucosidic linkages at the reducing end or at branch points of oligosaccharides[17], the α-(1→3) linkage was therefore located at the non-reducing end of the tetrasaccharides.

The proposed structure for sB_5 is significant because it indicates that some of the branched oligosaccharides derived from dextran carry 2-unit side-chains. If it be accepted that the B_5 fraction contains two components that arise from regions in the dextran molecule bearing 1-unit and 2-unit side-chains, respectively, then B_6, B_7, and higher fractions should also contain two components. The diagrams in Fig. 6 show that equal numbers of D-glucose residues would be released, and the same product, rB_5, would be obtained, from both the possible isomers of B_6 and B_7. It is only for

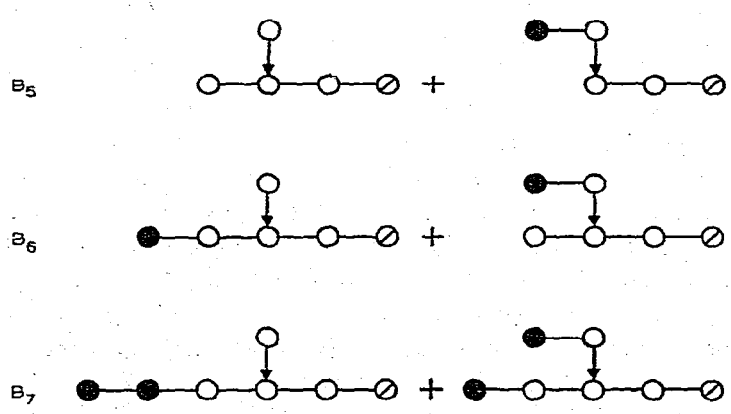


Fig. 6. Possible structures for branched oligosaccharide fractions derived from dextran. Each fraction may consist of two isomers, that contain 1-unit and 2-unit side-chains, respectively. ● D-Glucose residue that may be released by α-1,6-glucan glucohydrolase.

B_5 that the presence of two components can be revealed by the enzymic method, because each isomer gives a different result (0 or 1) for the number of D-glucose residues released.

The branched oligosaccharide products of *P. lilacinum* dextranase action on dextran B-512(F) should also contain isomers that bear 2-unit side-chains. A portion of the D-glucose released from B_6 by α-1,6-glucan glucohydrolase would then arise from 3^3-α-isomaltosylisomaltotetraose. Similarly, the single D-glucose residue released from B_7 might arise from 3^4-α-isomaltosylisomaltopentaose as well as from 3^4-α-D-glucosylisomaltohexaose.

The complete resistance of the branched pentasaccharide **8**, produced by *P. lilacinum* dextranase, to hydrolysis by α-1,6-glucan glucohydrolase indicated the absence of 3^3-α-isomaltosylisomaltotriose. This oligosaccharide could only be produced from dextran by an enzyme, such as *P. funiculosum* dextranase, that can hydrolyse the adjacent α-(1→6)-D-glucosidic linkage on the non-reducing side of the branch point. *P. lilacinum* dextranase does not hydrolyse this linkage to produce 3^3-α-D-glucosylisomaltotriose from dextran, and therefore the enzyme is unlikely to hydrolyse this same linkage when it occurs adjacent to a branch point in dextran that carries a 2-unit side-chain.

Catalytic oxidation of the dextrans produced by *L. mesenteroides* NRRL B-1415 and *L. dextranicum* NRRL B-1375 provided evidence that the branches consisted mainly of single D-glucose residues[18]. The action of *P. funiculosum* endo-dextranase was therefore expected to provide a pentasaccharide fraction containing a high proportion of rB_5. Tosylation of end groups in dextran from *L. mesenteroides* NCIB 2706 indicated that the side chains contained at least two D-glucose residues[8]; endo-dextranase might therefore have yielded sB_5 as the main pentasaccharide. However, the extent of hydrolysis of several B_5 fractions by α-1,6-glucan glucohydrolase (Table IV) indicated the possibility that all the dextrans may contain some 2-unit as well as 1-unit side-chains.

No evidence was found for the hydrolysis of the 2-unit side-chains in dextran B-512 during the preparation of α-1,6-glucan glucohydrolase limit dextran. The proportion of branched oligosaccharides in the products obtained from limit dextran (27.4%) was higher than in those from dextran (21.7%), because limit dextran contains all the α-(1→3) linkages that were in the original dextran. The figures are in agreement with the D-glucose released from dextran (22%) to provide limit dextran $[21.7 \times 100/(100-22) = 27.8]$. There was no change in the proportion of rB_5 in the pentasaccharide fraction, or in the proportion of B_4 in the branched products obtained by dextranase action on dextran and limit dextran. It was concluded that, although α-1,6-glucan glucohydrolase might release glucose from 2-unit side-chains in oligosaccharides, the enzyme had no action on these side chains when they occurred in dextran. A similar situation exists with another exo-glucanase, beta-amylase. This enzyme acts on amylopectin to give a limit dextrin in which 50% of the side chains contain three D-glucose residues. Yet beta-amylase rapidly hydrolyses 6-α-maltotriosyl-D-glucose to give maltose and isomaltose[19].

It is noteworthy that the structure we propose for sB_5, as well as the structures previously[12] deduced for B_4 and rB_5, are equivalent to those of the branched products of salivary alpha-amylase action on glycogen. When salivary alpha-amylase acts on glycogen or amylopectin, the comparatively long side-chains are decreased to one or two D-glucose residues. These short side-chains then appear in 6^3-α-D-glucosylmaltotriose (B_4), 6^3-α-D-glucosylmaltotetraose (fast B_5), and 6^3-α-maltosylmaltotriose (slow B_5)[20,21]. The higher, branched products in this series (B_6 and B_7) were also shown to consist of a mixture of isomers[20,22].

Although a proportion of long side-chains may also occur in dextran B-512(F) the majority of the side chains (85–90%) are short. Progressive shortening of the 10–15% of long side-chains in dextran, during the hydrolysis with *P. funiculosum* dextranase, to give 2-unit side-chains, could not account for the proportion of sB_5 found in the branched-pentasaccharide fraction. The results in Table V indicate that 2-unit side-chains may be common in dextrans.

The structures of all the branched oligosaccharides in the series obtained from glycogen (α-limit dextrins) were easily elucidated, because several enzymes were available for the hydrolysis of the α-(1→6)-D-glucosidic branch linkage. It seems probable that a definite proof of the structure and existence of multiple components in the branched oligosaccharides derived from dextran B-512(F), *S. bovis* dextran, and Birmingham dextran can be offered only when specific enzymes are found for the hydrolysis of the α-(1→3)-D-glucosidic branch linkages.

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