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Note

Frequency and distribution of branching in a dextran: an enzymic method*

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Several chemical and physical methods yield information on the frequency or relative content of non (1→6)-linkages in dextrans[1], and some of these methods (*e.g.*, methylation) unequivocally allocate such linkages as branch points. Other chemical methods have yielded information on the length of side chains[1]. Thus, for example, the commercially important B-512 dextran is known to have ~5% of (1→3)-α-D linkages present as branch points in the (1→6)-α-D-linked backbone chain, ~40% of the side chains consist of single D-glucose residues, 45% consist of an isomaltose residue, and the remainder contain more than two D-glucose residues[2]. However, no previous methods have given information on the distribution of branches along the backbone. We now report an enzymic approach to this problem.

An endo-dextranase from *Pseudomonas* UQM 733, known[3] as D_2, was selected for this purpose because its specificity is such that, for effective action, it requires a large number (more than 5) of successive (1→6)-linked α-D-glucopyranosyl units without branch points[4]. A sample of B-512 native dextran was treated with three successive additions of the enzyme until no further increase in reducing power occurred (Fig. 1), and the products were examined by quantitative t.l.c.[5] (see Table I). Linear oligosaccharides of the isomaltose series were detected with degrees of polymerisation (d.p.) ranging from 2 to 5, and branched oligosaccharides were also obtained with d.p. 4–11. The smaller, branched products B_5 and B_6 contained (p.m.r. spectroscopy) a single (1→3)-α-linkage, with the remainder being (1→6)-α-linkages[6]. We have assumed that the higher oligosaccharides (B_7–B_{11}) also contain a single (1→3)-α-linkage, and the relationship between their R_F and d.p. (Fig. 2) confirms this view. Similar inflexions to those in Fig. 2 for the linear oligosaccharide series have previously been observed by paper chromatography[7].

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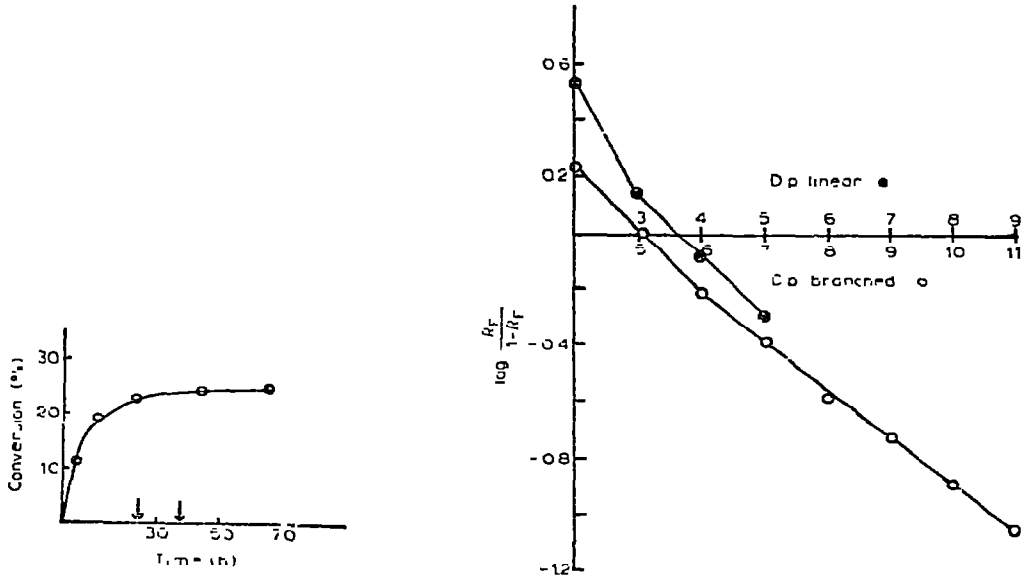


Fig 1 Conversion (%) into apparent glucose of B-512 dextran with enzyme D_2 at 33° Arrows indicate second and third additions of 0 016 enzyme unit

Fig 2 Relationship between d p and $\log R_F/(1-R_F)$ for isomaltose series of oligosaccharides R_f values are relative and not absolute, being calculated from an arbitrary value of 20 cm for the solvent front

The relative amount of each oligosaccharide product was determined by reference to standard curves[5], and the results are shown in Table I. The branched isomaltosaccharides, other than B_5 and B_6, were determined by reference to the linear isomaltosaccharide standard curve for d.p $n-1$, where n is the d.p. of the branched isomaltosaccharide The percentage branching was then determined by the following formulae.

Total weight (μg) of oligosaccharides present

$$= \sum_{n=2}^{n=5} W_{IM_n} + \sum_{n=4}^{n=11} W_{B_n},$$

Percentage of a particular oligosaccharide present (*e g* , IM_n)

$$= \frac{W_{IM_n} \times 100}{\sum_{n=2}^{n=5} W_{IM_n} + \sum_{n=4}^{n=11} W_{B_n}}$$

$$= \%\ IM_n \text{ or } B_n,$$

Percentage branching in dextran

$$= \sum_{n=4}^{n=11} \frac{\%\ B_n}{n};$$

Percentage conversion into apparent glucose

$$= \sum_{n=2}^{n=5} \frac{\%\ IM_n}{n} + \sum_{n=4}^{n=11} \frac{\%\ B_n}{n},$$

where W_{IM_n} is the weight (μg) of isomaltosaccharide present having d p $= n$, and W_{B_n} is the weight (μg) of branched isomaltosaccharide present having d p. $= n$.

The use of the formulae depends on the following assumptions. (*a*) The densitometer response after t l c of branched isomaltosaccharides of d.p $= n$ is the same as that of an equal quantity of a linear isomaltosaccharide of d p $= n - 1$. This assumption has been verified for B_5, IM_4 and B_6, IM_5. (*b*) The enzyme preparation does not contain any debranching activity. (*c*) The dextran is completely degraded by the enzyme. (*d*) There is only one branch unit per isomaltosaccharide produced.

The formulae can be used as shown to determine the percentage branching and also the conversion into apparent glucose, and gives the values listed in Table II. The close agreement between the enzyme hydrolysis–t l c method and the p m r method of branch-point determination indicates that the assumptions made in the application of the formula to B-512 native dextran are valid

TABLE I

ISOMALTOSACCHARIDES FORMED BY HYDROLYSIS OF B 512 NATIVE DEXTRAN WITH D_2

Isomaltosaccharide	*Quantity present (μg)*	*Relative amount (%)*	*Relative molar yield*
IM_2	2 2	3 7	1 85
IM_3	13 2	22 8	7 6
IM_4	12 3	20 9	5 2
IM_5	13 4	22 8	4 6
B_4	2 3	3 9	1 0
B_5	2 1	3 6	0 7
B_6	2 6	4 4	0 7
B_7	4 8	8 2	1 2
B_8	1 4	2 4	0 3
B_9	2 1	3 6	0 4
B_{10}	1 7	2 9	0 3
B_{11}	0 6	1 1	0 1

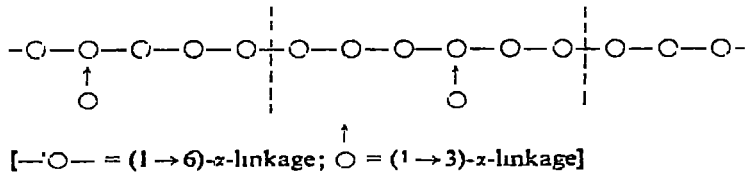
TABLE II

BRANCHING (%) AND CONVERSION (%) INTO APPARENT GLUCOSE OF B-512 NATIVE DEXTRAN

	Enzyme hydrolysis–t l c method	*P m r method*[6]	*Nelson–Somogyi method*
Branching (%)	4 7	4 6	
Conversion (%) into apparent glucose	24 0		25 4

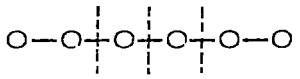
T l c. of the enzymic degradation products showed that the dextran was completely degraded to the oligosaccharides listed in Table I, and that no material

remained with d.p. greater than 11. The amount of branched oligosaccharides reaches a maximum at d.p. 7, and such products probably result from scissions such as.

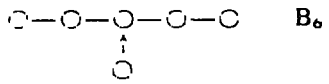


[—O— = (1→6)-α-linkage; O = (1→3)-α-linkage]

The enzyme hydrolyses IM_6 as follows

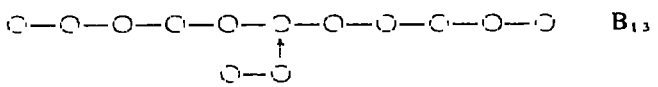


Assuming that D_2 can only produce chain scission where there are at least six contiguous main-chain units unbranched, the smallest, and largest, significant branched-oligosaccharides to be expected from the dextran degradation were:



B_6

and



B_{13}

All oligosaccharides of intermediate d.p would also be expected Since IM_5 is very slowly attacked by the enzyme to produce IM_{2-4}, it is probable that the prolonged degradation of the dextran has produced limited secondary attack, especially on the larger, primary branched-products, resulting in the distribution shown in Table I. However, the high yields of B_4 and B_5 were unanticipated, and cannot be explained on the basis of rate studies with linear oligosaccharides

It is not possible to reach any exact conclusions about the distribution of branches from the above results, but certain firm, general comments can be made. The majority of branch points are separated from the next branch point by six or more unsubstituted (1→6)-linked α-D-glucopyranose residues No regions in the dextran molecule contain "bunches" or local, high concentrations of branch points, because no significant product remains which is larger than d p 11. Finally, since the average separation of branch points must be less than 18 D-glucose residues, we conclude that the branch points are distributed along the backbone in a relatively regular manner. Our results would not be compatible with a statistically random distribution of branch points, since this would require a significant number to be separated by less than six D-glucose residues, and this, in turn, would result in the production of significant amounts of products of higher d.p. than those detected.

EXPERIMENTAL

Materials and methods. — The B-512 dextran (batch TG 2941) was supplied by Pharmacia AB, Sweden, and was the native dextran obtained directly from the *Leuconostoc* fermentation without hydrolysis or fractionation

The isomaltose oligosaccharides will be referred to as either IM_n or B_n, where IM refers to the linear oligosaccharide, B refers to the oligosaccharide with a single (1→3)-α-branch, and *n* refers to the d.p in both cases. IM_3–IM_9 were prepared by M Streamer[4], and the IM_2 was a gift from Dr H. Schiweck, Suddeutschenzucker-AG B_5 and B_6 were prepared in this laboratory by enzymic hydrolysis of B-512 native dextran, and their structures have been investigated by p m r. spectroscopy[6].

The enzyme used was an extracellular endo-dextranase (D_2) isolated and purified from the bacterium *Pseudomonas* UQM 733, as described earlier[3].

Hydrolysis of B-512 native dextran with D_2 enzyme. — B-512 Native dextran (~5 mg, accurately weighed) was dissolved in 0.9 ml of 0.02M citrate buffer (pH 5 5). The carbohydrate content of the solution was determined in duplicate on 0.1-ml fractions by the phenol-sulphuric acid method[8] To the remainder of the solution, 0 016 unit of D_2 enzyme was added: the solution was then saturated with toluene to prevent microbial growth, and incubated at 33°. The hydrolysis of the dextran was followed by withdrawing, in duplicate, 0 02-ml samples for determination of reducing power by the Nelson–Somogyi method[9]. After 24 and 38 h, respectively, additional amounts of 0 016 unit of enzyme were added. When the reaction had reached completion, a 0.05-ml sample was deionised with mixed ion-exchange resins [Amberlite IR-45(OH^-) and IR-120(H^+)], and 0 02 ml was subjected to quantitative t l c. for 19 h at 30°, in the continuous mode described earlier[5].

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REFERENCES

1 R. L Sidebotham, *Adran Carbohydr Chem. Biochem*, 30 (1974) 371–444
2 O. Larm, B Lindberg and S Svensson, *Carbohydr Res*, 20 (1971) 39–48
3 G N Richards and M Streamer, *Carbohydr Res*, 25 (1972) 323–332
4 M Streamer, Ph D Thesis, James Cook University of North Queensland, 1973
5 M T Covacevich and G N Richards, *J Chromatogr*, 129 (1976) 420–425
6 M T Covacevich, Ph D Thesis, James Cook University of North Queensland, 1976
7 E J Bourne, D H Hutson, and H Weigel, *Biochem J*, 86 (1963) 555–562
8 M Dubois, K A. Gilles, J K Hamilton, P. A Rebers, and F Smith, *Anal Chem*, 28 (1956) 350–356
9 N Nelson, *J Biol Chem*, 153 (1944) 375–380