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Note

Mode of action of dextranase D_2 from *Pseudomonas* UQM 733 on oligosaccharides*

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Well-characterised, pure dextranases offer great advantages to the study of the fine structure of dextrans. However, although there are many recent reports of dextranases induced in various fungi and bacteria, few workers have been concerned with the rigorous purification and subsequent characterisation of the mode of action of these enzymes[1].

We have described[2,3] the isolation of two extracellular endo-dextranases (D_1 and D_2) from a species of *Pseudomonas* (UQM 733). The mode of action of pure dextranase D_1 has been studied and a model for its catalytic centre proposed. We now describe similar work with dextranase D_2, which differs from D_1 in a number of ways.

Purification[2] of D_2 by gel-permeation chromatography gave a product which showed two protein bands on gel electrophoresis. Only the major band had dextranase activity. It was not possible to remove the non-dextranase protein from D_2, but no glycanase activity was detected, other than that against (1 → 6)-α-D-glucan, for a wide range of polysaccharide types. The preparation is also free from activity against sucrose, maltose, and cellobiose. Thus, D_2 appears to be suitable for use in structural studies on dextrans, and its specificity and action pattern against a series of oligosaccharides have been investigated.

The time course of degradation of isomaltosaccharides of d.p. 7–12 (IM_7–IM_{12}) is shown in Fig. 1, and the products are indicated in Table I. The rate of degradation and the percentage increase in reducing power (*i.e.*, scissions per molecule) increased with increase in d.p. 7 → 12, but a maximum was not attained. Although IM_2–IM_6 showed no measurable increase in reducing power on incubation with D_2, p.c. of the IM_5 and IM_6 digests indicated slight attack by the enzyme during 3 days. Each substrate was degraded slightly to IM_2, IM_3, and IM_4 with trace amounts of glucose, whereas IM_6 also gave small proportions of IM_5. Little

*Studies on Dextranases: Part VI. For Part V, see Ref. 7.

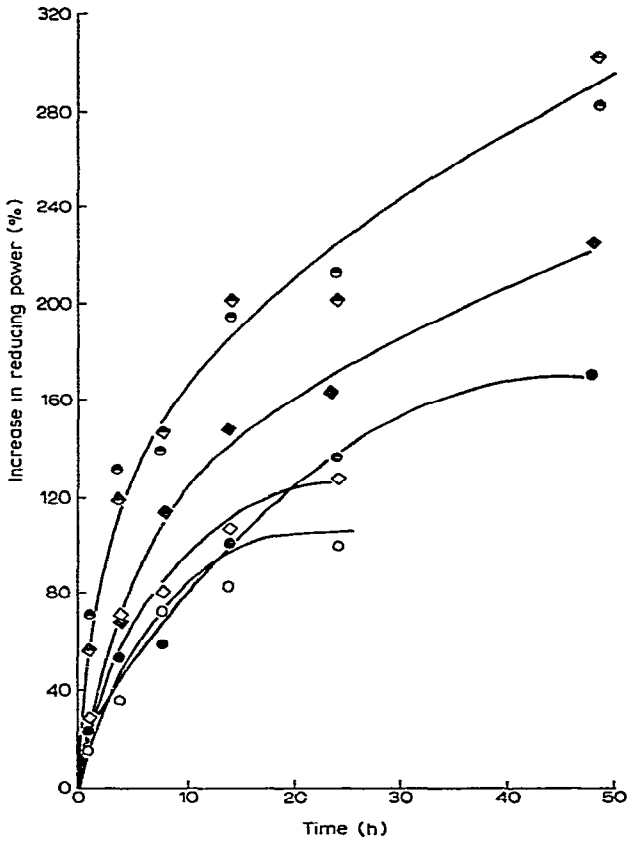


Fig. 1. Degradation of oligosaccharides by dextranase D_2 at 33°: —○— IM_7, —●— IM_8, —◇— IM_9, —◆— IM_{10}, —◆— IM_{11}, —◓— IM_{12}; 3.8 ±0.3mM; D_2 2.3 × 10^{-2} unit.

or no glucose, isomaltose, or oligosaccharides of d.p. one or two less than the substrate were produced from the action of D_2 against substrates of d.p. >7 and thus, in contrast to D_1, D_2 did not hydrolyse glycosidic linkages close to either end of the substrate chain. The D_2 enzyme showed a marked tendency to hydrolyse centrally located glycosidic linkages. This property was most obvious on degradation of IM_{10}, IM_{11}, and IM_{12}, where no significant products of d.p. <3 were evident. There was no evidence for any synthetic activity such as was observed with D_1.

Fig. 2 shows the yields of products formed on degradation of IM_{10} with D_2. IM_5 was produced most rapidly (84 × 10^{-3} μmol.h^{-1}) and must derive from hydrolysis of the central glycosidic linkage. IM_4 and IM_6 were produced initially at the same rate (combined rate, 65 × 10^{-3} μmol.h^{-1}), which was greater than that for IM_7 and IM_3 (combined rate, 25 × 10^{-3} μmol.h^{-1}). Hydrolysis of the central glycosidic linkage was therefore the preferred mode of attack of D_2 on IM_{10}, followed by hydrolysis of other linkages at a rate decreasing with distance from the central linkage.

TABLE I

PRODUCTS OF DEGRADATION OF ISOMALTOSE OLIGOSACCHARIDES[a] (3.8 ±0.3mM) BY DEXTRANASE D_2 (2.3 × 10^{-2} UNIT)

Products	*IM*$_2$		*IM*$_3$		*IM*$_4$		*IM*$_5$		*IM*$_6$		*IM*$_7$		*IM*$_8$		*IM*$_9$		*IM*$_{10}$		*IM*$_{11}$		*IM*$_{12}$	
D-Glucose	—	—[c]	—	—[c]	—	—[c]	—	trace[c]	—	1[c]	—	trace	—	—	—	—	—	—	—	—	—	—
IM_2	4	4	—	—	—	—	—	1	1	1	1	1	trace		—	trace	—	—	—	—	—	trace
IM_3			4	4	—	—	—	1	1	2	2	2	1	2	1	1	1	2	1	1	1	1
IM_4					4	4	—	1	1	2	2	2	1	2	2	3	2	3	2	3	2	3
IM_5							4	3	—	1	1	1	1	2	2	3	2	3	3	3	2	3
IM_6									4	2	—	—	—	—	1	1	2	2	3	3	2	trace
IM_7											4	1	—	—	—	—	1	—	2	—	2	—
IM_8													3	1	—	—	—	—	1	—	trace	—
IM_9															2	1	—	—	—	—	—	—
IM_{10}																	2	1	—	—	—	—
IM_{11}																			1	1	—	—
IM_{12}																					1	—

[a]IM_x connotes an isomaltosaccharide containing x glucose residues. [b]The figures represent a visual estimation of the amounts after p.c. The pair of numbers in each column refers to samples taken after 4 h and 2 days, respectively. [c]Samples taken after 3 days.

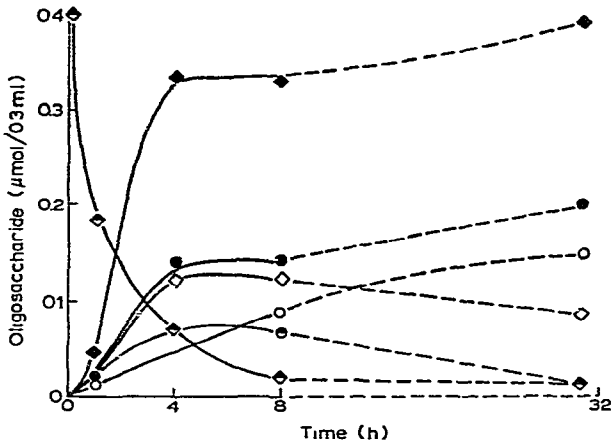


Fig. 2. Degradation of IM_{10} (2·7mM) by dextranase D_2 (13.7×10^{-2} unit initially, $6\,5 \times 10^{-2}$ unit added after 24 h): —○— IM_3, —●— IM_4, —◆— IM_5, —◇— IM_6, —⊖— IM_7, —◈— IM_{10}.

TABLE II

DEGRADATION OF REDUCED, TRITIATED IM_{10} (6mM) WITH DEXTRANASE D_2 (2.3×10^{-2} UNIT)

Reduced, tritiated oligosaccharide	*Counts/min (% total)*	*Frequency of production*[a]
IM_2-itol	1	0.01
IM_3-itol	13	0.13
IM_4-itol	23	0.23
IM_5-itol	41	0.41
IM_6-itol	15	0.15
IM_7-itol	4	0.04
IM_{10}-itol	3	

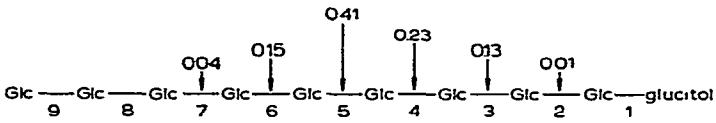
[a]Counts (%) in each oligosaccharide product/total counts (%) for oligosaccharide products.

In addition (*cf.* Table I), there was some secondary hydrolysis of the primary products from IM_{10}, *e.g.*, IM_7 and IM_6, as indicated by their decrease in concentration after prolonged incubation times.

It was not possible to determine whether or not the rates of bond scission in IM_{10} were symmetrical on either side of the central bond because, for example, scission of bond 4 (see below) gives the same products as scission of bond 6. However, the fit of the oligosaccharide substrates into the enzyme binding-site is likely to be unidirectional. In order to resolve this point, IM_{10} was reduced with sodium borotritiide, thereby converting the reducing end-group into D-glucitol-*1-t*.

Table II shows the distribution of radioactive products obtained on degradation of reduced IM_{10} by D_2, and the relative frequency of production of each as calculated by the method of Robyt and French[4]. The calculation assumes that all of the radioactive products are derived from the primary scission of reduced IM_{10}. This assumption is considered valid, because the largest, initial, tritiated product (reduced IM_7) is degraded[5] at half the rate of IM_7 which, in turn, is degraded slowly compared to IM_{10}. Thus, the presence of an alditol group at the reducing end of isomaltosaccharides of d.p. $\leqslant 7$ renders the molecules relatively stable to attack by D_2.

The scission of reduced IM_{10} by D_2 can be represented diagramatically as follows:



Thus, bond 5 was hydrolysed most rapidly.

The alditol grouping in reduced IM_{10} did not prevent formation of an active enzyme–substrate complex; in fact, more bond cleavage occurred on the side towards the alditol group. Thus, the fit of IM_{10} and reduced IM_{10} in the active site appears to be similar and there is a similar asymmetrical pattern of rates of bond scission. A more complex asymmetry of distribution of hydrolysis rates along the oligosaccharide chain has been observed by Walker and Dewar[6] in the degradation of substrates, labelled at the non-reducing end, by *Penicillium lilacinum* dextranase.

Whereas dextranase D_1 hydrolyses smaller isomaltosaccharides ($\geqslant IM_5$) and reaches a maximum rate of attack with IM_8, D_2 shows no maximum rate of attack with oligosaccharides of d.p. 7–12, with IM_7 being the smallest isomaltosaccharide to be hydrolysed at a significant rate. Both dextranases show a preference for hydrolysis of glycosidic linkages towards the reducing end of the susceptible oligosaccharides that are degraded at less than the maximum rate. This pattern reflects the nature of the active site which, for D_1, has been postulated[3] to consist of six glucose-binding subsites arranged asymmetrically about the catalytic site. With oligosaccharides of d.p. >6, the subsites can be filled in more than one way and hydrolysis is more random, with the centrally located linkages being hydrolysed most frequently.

It is not possible to postulate an exact model for the active centre of D_2, as the largest oligosaccharide available, IM_{12}, did not indicate that a maximal rate of hydrolysis had been attained. However, the available results suggest that D_2 has a much larger active centre than does D_1 and that ten or more glucose-binding subsites could be involved. The results of degradation of reduced IM_{10} suggest that the catalytic site is asymmetrically placed within the binding subsites, probably towards the reducing end. On this basis, and assuming that the active site will not accommodate a branch point in a dextran molecule, it is unlikely that D_2 will hydrolyse dextran molecules at a significant rate, except in regions containing an unbranched sequence of at least seven (1 → 6)-linked α-D-glucosyl residues. This conclusion

has been used to derive information on the distribution of branch points in B-512 dextran[7].

EXPERIMENTAL

General methods and the preparation of substrates have been described earlier[2,3].

Enzymic hydrolysis of isomaltose oligosaccharides. — A solution (0.5 ml) of the oligosaccharide (3.8mM, d.p. 2–12) and dextranase D_2 (2.3×10^{-2} unit) in 54mM sodium citrate buffer (pH 5.5) was kept at 33°, and reducing power was monitored by the Nelson–Somogyi method[8] and paper chromatography (p.c.).

For the quantitative determinations, a solution (2.6 ml) containing 54mM citrate buffer (pH 5.5), D_2 (13.7×10^{-2} unit), and IM_{10} (1.35mM) was kept at 33°, and 300-μl samples were removed after 1, 4, and 8 h. After 24 h, more enzyme (6.5×10^{-2} unit) was added, and the final sample was removed after a further 8 h. The relative amounts of the different oligosaccharides in each sample were determined as described previously.

Enzymic hydrolysis of tritiated, reduced isomaltodecaose. — A solution (0.5 ml) containing 54mM citrate buffer (pH 5.5), 6.0mM tritiated, reduced isomaltodecaose (9,000 counts.min^{-1}.μmol^{-1}), and D_2 (2.3×10^{-2} unit) was kept at 33°, and 100-μl samples were removed after 1 and 24 h. The relative amounts of radioactive products were determined as described previously.

Activity of dextranase D_2 against other poly- and di-saccharides. — D_2 (2.1×10^{-2} unit) was incubated with 1% solutions of potato starch, amylose, amylopectin, glycogen, pullulan, carboxymethylcellulose, laminaran, nigeran, hemicellulose B from sugar cane, sucrose, cellobiose, or maltose in 54mM citrate buffer (pH 5.5) at 40° for 24 h. The reducing power of the solutions was then measured, and compared with the control solutions lacking enzyme.

REFERENCES

1 R. L. Sidebotham, *Adv. Carbohydr. Chem. Biochem.*, 30 (1974) 371–444.
2 G. N. Richards and M. Streamer, *Carbohydr. Res.*, 25 (1972) 323–332.
3 G. N. Richards and M. Streamer, *Carbohydr. Res.*, 32 (1974) 251–260.
4 J. F Robyt and D French, *J. Biol. Chem.*, 245 (1970) 3917–3927.
5 M. Streamer, Ph.D. Thesis, James Cook University of North Queensland, 1973.
6 G. J. Walker and M. D. Dewar, *Carbohydr. Res.*, 39 (1975) 303–316.
7 M. T. Covacevich and G. N. Richards, *Carbohydr. Res.*, 54 (1977) 311–315.
8 N. Nelson, *J. Biol. Chem.*, 153 (1944) 375–380.