DEGRADATION OF ALPHA-LINKED D-GLUCO-OLIGOSACCHARIDES AND DEXTRANS BY AN ISOMALTO-DEXTRANASE PREPARATION FROM ARTHROBACTER GLOBIFORMIS T6*

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SUMMARY: Further studies were made on the action of isomalto-dextranase from Arthrobacter globiformis (T6) using gluco-oligosaccharides and dextrans. This enzyme releases isomaltose from some oligosaccharides by splitting not only the (1→6)-α-linkage but also (1→2)-α-, (1→3)-α- and (1→4)-α- linkages. Its exo-lytic mode of action was confirmed using isomaltohexaitol. The enzyme was also found to recognize both O-α-D-Glcp-(1→2 and 3)-O-α-D-Glcp-(1→6)-D-Glc as isomaltose and to split these trisaccharides from a gluco-tetrasaccharide and dextrans. A possible mechanism of action of this isomalto-dextranase on dextrans is proposed.

In two previous papers (1, 2), we reported a novel dextranase differing from those so far known. This dextranase, tentatively named G_2- or isomalto-dextranase, releases isomaltose units successively from the non-reducing terminals of dextrans and isomalto-oligosaccharides. The bacteria producing this G_2-dextranase extracellularly, strain T6, was recently identified as Arthrobacter globiformis. It will be described elsewhere. This paper reports further studies on the action of Arthrobacter G_2-dextranase using isomalto-oligosaccharides, variously α-linked gluco-oligosaccharides and dextrans. Results showed that this dextranase has an unexpectedly broad substrate specificity.

MATERIALS AND METHODS

A solution of purified Arthrobacter G_2-dextranase containing ca. 25 units of activity/ml was prepared as described before (2). Dextrans T40, T2000, B512, B1397 and N4 were the same as those used before (1-5). Dextran NRRL B1355 and panose were gifts from Dr. A. Jeanes of the Northern Regional Research Laboratories, Peoria, Ill., U. S. A. and α-isomaltosyl-(1→3)-D-

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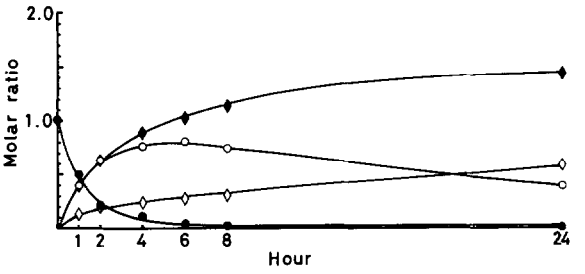

Fig. 1. Molar ratio of reaction products of isomaltohexaitol liberated by G_2-dextranase. Isomaltose, ◆ ; isomaltitol, ◇ ; isomaltotetraitol, ○ ; isomaltohexaitol, ● .

glucose was kindly given by Dr. K. Matsuda, Tohoku University, Sendai, Miyagi, Japan. The other trisaccharides and isomalto-oligosaccharides shown in Table 1 were prepared by acetolysis or acid hydrolysis of dextrans (3, 6, 7). Borohydride-reduced forms of oligosaccharides were prepared as described elsewhere(3).

A tetrasaccharide, tentatively named oligosaccharide A, was obtained by preparative paper chromatography from an enzymic digest of dextran T40. The endo-lytic dextranase used for this digestion was supplied from the Research Foundation for Microbial Diseases of Osaka University, Kan'onji, Kagawa, Japan. This oligosaccharide A gave glucose, isomaltose and isomaltotriose as the main products upon oxalic acid hydrolysis under the conditions reported by Bourne et al. (8). It was concluded to be a tetrasaccharide because the molar ratio of glucose in it before and after borohydride-reduction was 4.00:3.08. After periodate-oxidation 26% of its glucose remained, indicating that one of the three linkages is probably (1→3) and that this linkage is not in the reducing end. On gas chromatographic analysis of the methylated sugar components of oligosaccharide A, 2, 3, 4, 6-tetra-O-methyl-, 2, 4, 6-tri-O-methyl- and 2, 3, 4-tri-O-methyl-glucoses were found in a ratio of 1.00:0.84:1.74. Therefore oligosaccharide A was identified as O-α-D-Glcp-(1→3)-O-α-D-Glcp-(1→6)-O-α-D-Glcp-(1→6)-D-Glc.

Methylation, paper chromatography and sugar analysis (automatic, liquid chromatography) were performed as described before (3, 4, 9). The action of G_2-dextranase was examined in 0.1 M acetate buffer, pH 5, at 37°C.

RESULTS AND DISCUSSION

Action of G_2-dextranase on isomaltohexaitol. The reaction mixture (120 µl) contained 300 µg of isomaltohexaitol and 10 µl of G_2-dextranase solution. Samples were taken at intervals for analysis of sugar. The results in Fig. 1 show that initially isomaltohexaitol is mainly split into isomaltose and isomaltotetraitol, then the accumulated isomaltotetraitol gradually decreases with increase in the two final products, isomaltose and isomaltitol. These results provide direct evidence that G_2-dextranase splits isomaltose from the non-reducing end of the hexaitol and that the enzyme can attack dextrans exo-lytically, as previously suggested (1).

Table 1 Actions of G_2-dextranase on various oligosaccharides

Substrate	Products identified
Isomaltotriose	Isomaltose, glucose
Isomaltotetraose	Isomaltose
Isomaltopentaose	Isomaltose, glucose
Isomaltohexaose	Isomaltose
Isomaltoheptaose	Isomaltose, glucose
Isomaltotriitol	Isomaltose, glucitol
O-α-Isomaltosyl-(1→2)-D-Glc	Isomaltose, glucose
O-α-Isomaltosyl-(1→3)-D-Glc	Isomaltose, glucose
O-α-Isomaltosyl-(1→4)-D-Glc	Isomaltose, glucose
O-α-D-Glcp-(1→2)-O-α-D-Glcp-(1→6)-D-Glc	-*
O-α-D-Glcp-(1→3)-O-α-D-Glcp-(1→6)-D-Glc	-
O-α-D-Glcp-(1→4)-O-α-D-Glcp-(1→6)-D-Glc	-
O-α-D-Glcp-(1→2)-O-[α-D-Glcp-(1→6)]-D-Glc	-
O-α-D-Glcp-(1→3)-O-α-D-Glcp-(1→6)-O-α-D-Glcp-(1→6)-D-Glc	O-α-D-Glcp-(1→3)-O-α-D-Glcp-(1→6)-D-Glc, glucose

* - none.

Action of G_2-dextranase on isomalto-oligosaccharides and variously α-linked gluco-oligosaccharides. Volumes of 120 µl of reaction mixture containing 200 µg of substrate and 40 µl of enzyme solution were incubated overnight and then subjected to paper chromatography and sugar analysis. The results in Table 1 show that the isomalto-oligosaccharides were degraded to isomaltose and glucose, or isomaltose alone, as expectedly. Among the trisaccharides containing two kinds of linkages, three O-α-isomaltosyl-(1→2, 3 and 4)-glucoses were each split into isomaltose and glucose, indicating that

the enzyme can attack (1→2), (1→3), (1→4) or (1→6) α-isomaltosyl linkages. However, the other four O-α-glucosyl-isomaltoses tested were not attacked. The most remarkable result in Table 1 is that the tetrasaccharide was hydrolyzed to a trisaccharide, instead of isomaltose, and glucose. This trisaccharide was identified as O-α-D-Glcp-(1→3)-O-α-D-Glcp-(1→6)-D-Glc. Although G_2-dextranase was originally reported to release only isomaltose from dextrans, it is possible that this particular trisaccharide was released as final hydrolysis product of dextran, because it can be regarded as a modified isomaltose. We confirmed the presence of this trisaccharide and also another one in digests of dextrans by this dextranase preparation, as described below.

Detection and identification of trisaccharides released from dextrans by G_2-dextranase. Dextrans N4, B1355 and B1397 were incubated with G_2-dextranase for several days to achieve extensive hydrolysis. Then the reaction mixtures were dialyzed against water, and the outer solutions were deionized with ion-exchange resins, concentrated and subjected to paper chromatography. A faint spot(s) was detected in the region of trisaccharide, in addition to the major spot of isomaltose. The material(s) in the minor spot(s) was eluted with water and compared with reference trisaccharides by paper and liquid chromatographies. The pattern of the digest and the locations of reference trisaccharides on liquid chromatography are shown in Fig. 2. The trisaccharide from dextrans N4 and B1355 was identified as O-α-D-Glcp-(1→3)-O-α-D-Glcp-(1→6)-D-Glc while those from dextran B1397 were identified as O-α-D-Glcp-(1→2 and 3)-O-α-D-Glcp-(1→6)-D-Glc. Thus the G_2-dextranase degrades dextrans from their non-reducing terminals, releasing not only isomaltose but also these trisaccharides. These trisaccharides must be recognized by G_2-dextranase as modified isomaltoses, as suggested by the results on a tetrasaccharide shown in Table 1. G_2-Dextranase probably does not recognize the other kinds of trisaccharides, O-α-D-Glcp-(1→2 and 3)-O-[α-D-Glcp-(1→6)]-D-Glc as modified isomaltoses because none of these were detected in the digests of dextrans.

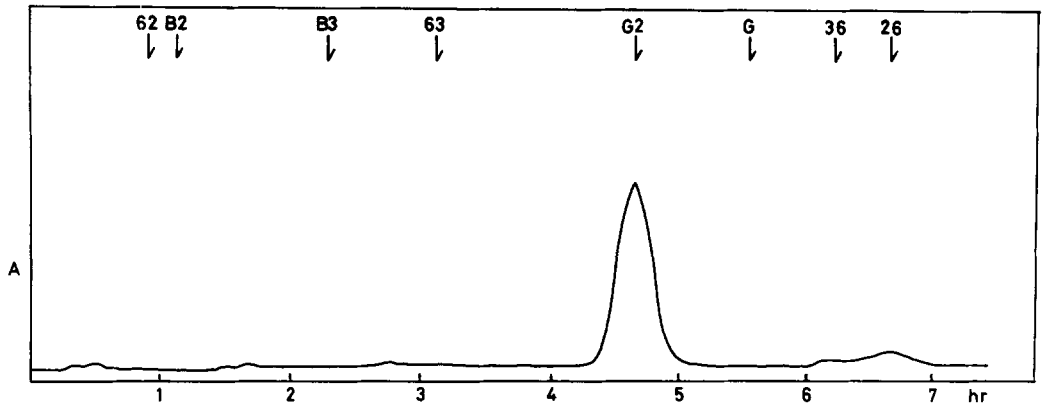

Fig. 2. Pattern of the dialyzable fraction of the digest of dextran B1397 with dextranase and locations of reference oligosaccharides on liquid chromatography. Chromatographic conditions were the same as condition (ii) described previously (9). Arrows indicate locations of various oligosaccharides. O-α-D-Glcp-(1→6)-O-α-D-Glcp-(1→2)-D-Glc, 62; O-α-D-Glcp-(1→2)-O-[α-D-Glcp-(1→6)]-D-Glc, B2; O-α-D-Glcp-(1→3)-O-[α-D-Glcp-(1→6)]-D-Glc, B3; O-α-D-Glcp-(1→6)-O-α-D-Glcp-(1→3)-D-Glc, 63; O-α-D-Glcp-(1→3)-O-α-D-Glcp-(1→6)-D-Glc, 36; O-α-D-Glcp-(1→2)-O-α-D-Glcp-(1→6)-D-Glc, 26; isomaltose, G2; glucose, G.

From the above results, it is possible to depict the mechanism of action of G_2-dextranase on polyglucosyl chains of dextran molecules, which have a ramified structure with both (1→2) and (1→3) branches in B1397 (4) and with only (1→3) branches in B512, N4 and B1355 (10-12). Fig. 3 shows an example. α-Isomaltosyl linkages can be split by G_2-dextranase. After complete hydrolysis of straight glucosyl chains the end of the chain becomes O-α-D-Glcp-(1→2 or 3)-O-α-D-Glcp-(1→6)-α-D-Glcp- and trisaccharides of these kinds are split exposing a new straight chain to the enzyme. This mechanism fully explains the unusually high extent of hydrolysis of dextrans by this enzyme. For instance under suitable conditions G_2-dextranase causes about 50% hydrolysis of dextrans B512, T2000 and N4, whereas an exo-lytic dextranase (dextranglucosidase) from Streptococcus mitis which releases glucose units was reported only to cause 23-25% hydrolysis of dextran B512 (13). However, if a straight chain part is incompletely removed so that a penultimate glucose residue is flanked with a (1→2 or 3)-α-glucosyl stub, the action of G_2-

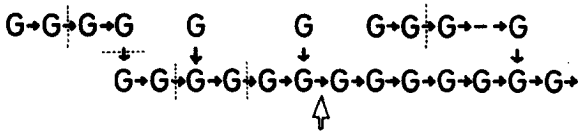

Fig. 3. Action of G_2-dextranase on dextrans.
→ , (1→6)-α- linkage; ↓ , (1→2)-α- or (1→3)-α- linkage;
⋮ , linkage split; ⇧ , linkage not split.

dextranse stops. This may be why, although it has relatively broad specificity, it leaves a limit dextrandextrin on hydrolysis of dextrans (1). G_2-Dextranase can probably act on side chains consisting of (1→6)-α-links, releasing isomaltose from non-reducing ends. However, it is still unknown how close to the junctions of these side chains it acts.

REFERENCES

1. Sawai, T., Toriyama, K. and Yano, K. (1974) J. Biochem. 75, 105-112.
2. Sawai, T. and Niwa, Y. (1975) Agr. Biol. Chem. 39, 1077-1083.
3. Sakakibara, K., Torii, M., Misaki, A. and Miyaji, H. (1972) Carbohyd. Res. 25, 443-451.
4. Miyaji, H., Misaki, A. and Torii, M. (1973) Carbohyd. Res. 31, 277-287.
5. Torii, M., Sakakibara, K. and Kabat, E. A. (1973) J. Immunol. 110, 951-958.
6. Schlossman, S. F. and Kabat, E. A. (1962) J. Exp. Med. 116, 535-552.
7. Yamauchi, F. and Matsuda, K. (1969) Agr. Biol. Chem. 33, 103-109.
8. Bourne, E. J., Huston, D. H. and Weigel, H. (1963) Biochem. J. 86, 555-562.
9. Torii, M. and Sakakibara, K. (1974) J. Chromatogr. 96, 255-257.
10. Lindberg, B. and Svensson, S. (1968) Acta Chem. Scand. 22, 1907-1912.
11. Miyaji, H., Misaki, A. and Torii, M. (1971) Abstr. Paper Ann. Meeting Agr. Chem. Soc. Japan, p. 276.
12. Misaki, A., Goldstein, I. J., Torii, M. and Sawai, T. (1975) Seikagaku 47, 743.
13. Walker, G. J. and Pulkownik, A. (1973) Carbohyd. Res. 29, 1-14.