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Note

The action of malted-barley alpha-amylase on amylopectin*†

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The action of alpha-amylase on amylopectin and glycogen gives maltose, α-dextrins, and either D-glucose or maltotriose. The exact composition of the alpha-amylolysate is dependent on the concentration and source of the enzyme. During the first stage of alpha-amylolysis, maltotriose is produced, which is hydrolysed to maltose and D-glucose if the enzyme concentration is increased[2]. At the same time, branched oligosaccharide α-dextrins containing side chains of three or four D-glucose residues may yield maltose and an α-dextrin containing side chains of only one or two D-glucose residues, during this second-stage alpha-amylolysis.

Alpha-amylases from different sources show some variation in their ability to hydrolyse (1→4)-α-D-glucosidic linkages in the vicinity of the (1→6)-inter-chain linkage (see Fig. 1)[3]. The smallest α-limit dextrin produced by human salivary[4], *Aspergillus oryzae*[5], and porcine pancreatic[5] alpha-amylases is the tetrasaccharide 6^3-α-D-glucosylmaltotriose (Fig. 2, structure 2), whereas, with *Bacillus subtilis* alpha-amylase, the pentasaccharide 6^2-α-maltosylmaltotriose (Fig. 2, structure 3) is produced[6]. Malted-rye alpha-amylase also gave 6^3-α-D-glucosylmaltotriose as the smallest α-limit dextrin derived from amylopectin[7]. However, malted-barley alpha-amylase has been reported[8] to give the trisaccharide panose (Fig. 2, structure 1) as the smallest α-limit dextrin, which would imply the hydrolysis of linkages C, F, and H (Fig. 1). During our study of the malted-

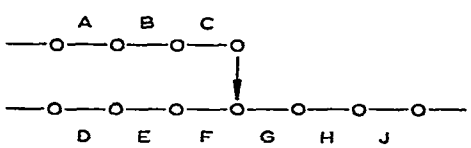

Fig.1. Segment of amylopectin near an inter-chain linkage. Key: o represents a D-glucosyl residue; ↓ represents a (1→6)-α-D-glucosidic inter-chain linkage; — represents a (1→4)-α-D-glucosidic linkage.

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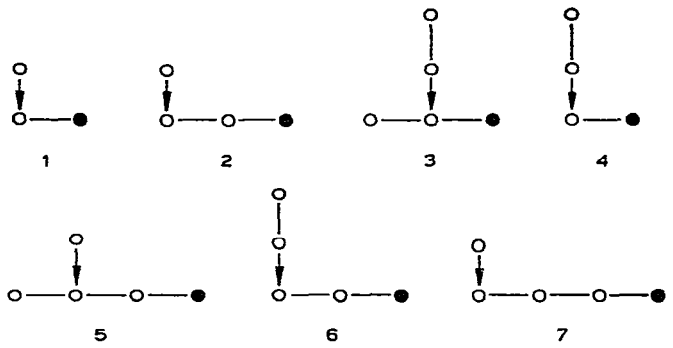

Fig.2. Partial formulae of possible α-limit dextrins. Key: as Fig. 1, and • represents a reducing D-glucose residue.

rye enzyme, a preliminary, comparative study of the malted-barley alpha-amylase was also made[7], and paper-chromatographic analysis indicated the absence of panose, but the presence of 6^3-α-D-glucosylmaltotriose as the smallest α-limit dextrin derived from amylopectin during second-stage hydrolysis. We now report confirmation of these results by methylation analysis. The general analytical methods used were as described previously[7].

Alpha-amylase was prepared from malted-barley flour (var. Golden Promise, 1972 harvest) by extraction with 0.2% aqueous calcium chloride, heating the extract at pH 5.6 to 70° for 35 min (to inactivate beta-amylase), fractionation with acetone at 0°, and precipitation of the enzyme with glycogen. The final enzyme preparation had a specific activity of 1,500 units/mg of protein (for assay, see Ref. 7), which represents a 230-fold purification. The overall procedure was similar to that developed by Greenwood and his co-workers[9], and used in the study of malted-rye alpha-amylase[7].

In preliminary experiments, the enzyme (3 units) was incubated with rat-liver glycogen or waxy-maize starch (~ 50 mg) at pH 5.5 and 37° in a total volume of 10 ml. The alpha-amylolysis limits after 60 h were 72 and 84%, respectively, and were not increased by the addition of further enzyme. Paper-chromatographic analysis showed the presence of large proportions of glucose and maltose in both digests, with traces of maltotriose, and small proportions of oligosaccharides having the mobility of tetra-, penta-, hexa-, and hepta-saccharides, respectively. Panose was absent.

In a larger scale digest, waxy-maize starch (1.0 g) was incubated with alpha-amylase (12 units) for 72 h, giving 82% conversion into apparent maltose. The enzyme was inactivated by heating. There was no further increase in reducing power when more alpha-amylase was added, and incubation was continued for 54 h. The digest was deionised, concentrated, and fractionated by preparative chromatography on Whatman No. 17 paper with ethyl acetate–pyridine–water (10:4:3). The oligosaccharides were purified by chromatography on Whatman No. 1 paper, to give ~ 20 mg of the suspected tetra- and penta-saccharides. Careful examination of the trisaccharide region of the chromatograms yielded only traces of maltotriose.

The tetrasaccharide was reduced with potassium borohydride, and then partially hydrolysed with acid. Paper chromatography showed the presence of glucose, glucitol,

maltose, maltitol, and maltotri-itol in the hydrolysate. The presence of the latter alcohol eliminates 4 (Fig. 2) as a possible structure.

The tetrasaccharide was then methylated by using a modification[10] of the Hakomori method[11], in which the oligosaccharide was subjected to three successive treatments with sodium methylsulphinylmethanide and methyl iodide. Control experiments with the normal Hakomori method, or the Kuhn method of methylation[12], did not give complete methylation of maltotriose and panose, and the recovery was low. The methylated tetrasaccharide was hydrolysed and derivatised in the normal way, and the mixture of alditol acetates was examined by g.l.c. with a Pye Series 104 Gas-Chromatograph (3% of OV-225 on Gas Chrom Q, at 200°, with nitrogen as the carrier gas). The products were the alditol acetates of 2,3,4,6-tetra-, 2,3,4-tri-, and 2,3,6-tri-*O*-methyl-D-glucose in yields of 26, 24 and 50 mol %. These results, together with those of the partial hydrolysis, characterise the tetrasaccharide as 6^3-α-D-glucosylmaltotriose (2, Fig. 2). This limit dextrin would arise from the hydrolysis of linkages C, F, and J (Fig. 1).

The second oligosaccharide was examined by methylation analysis. The products were 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methyl-D-glucose in yields of 41, 41, and 19 mol %. The oligosaccharide was not attacked by pullulanase[7] and therefore contains a single D-glucosyl group as a side chain. On the assumption that this pentasaccharide is related to the tetrasaccharide, these results characterise the α-limit dextrin as 6^3-α-D-glucosylmaltotetraose (5, Fig. 2), and eliminate 6^3-α-maltosylmaltotriose and 6^4-α-D-glucosylmaltotetraose (6 and 7, Fig. 2) as possible structures.

The third oligosaccharide is tentatively identified as 6^3-α-maltosylmaltotriose (slow B5)[7] (6, Fig. 2), because it is hydrolysed by pullulanase to give maltose and maltotriose. The identity of the two pentasaccharides provides further evidence that linkages G and H (Fig. 1) are resistant to malted-barley alpha-amylase.

We therefore conclude that the alpha-amylase from malted barley is similar to that from malted rye[7], human saliva[4], and porcine pancreas[5], in producing the tetrasaccharide 6^3-α-D-glucosylmaltotriose as the smallest α-limit dextrin from amylopectin.

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