The Specificity of Cereal Limit Dextrinases

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(Received 4 December 1969)

Limit dextrinase is an α -(1 $\rightarrow$ 6)-glucosidase, present in certain cereals, that acts on branched oligosaccharide α -dextrins (Kneen, 1945; Hopkins, 1959); these are formed, together with maltose and either glucose or maltotriose, during the α -amylolytic degradation of amylopectin or glycogen. The limit dextrinase from germinated barley was purified by Manners & Rowe (1968) and shown qualitatively to hydrolyse α -(1 $\rightarrow$ 6)-glucosidic linkages in a range of α -dextrins containing maltosyl or maltotriosyl side chains, but to have no action on substrates having α -glucosyl side chains, or on amylopectin. This work has now been extended to include quantitative studies of the rate of hydrolysis of various α -dextrins.

During this work, and similar studies on the limit dextrinase from ungerminated oats, amylopectin β -limit dextrin and pullulan were tested as possible substrates. These polysaccharides also contain maltosyl or maltotriosyl units. Pullulan is an extracellular polysaccharide synthesized by *Pullularia pullulans* that consists essentially of an unbranched chain of (1 $\rightarrow$ 6)-linked α -maltotriose units (Bender & Wallenfels, 1961). This glucan is degraded to maltotriose by enzyme preparations from certain bacteria, e.g. *Aerobacter aerogenes*, *Escherichia intermedia* and *Streptococcus mitis* (Abdullah *et al.* 1966; Ueda & Nanri, 1967; Walker, 1968), which specifically hydrolyse the α -(1 $\rightarrow$ 6)-glucosidic linkages. The same enzymes also hydrolyse the outermost α -(1 $\rightarrow$ 6)-glucosidic linkages in glycogen and amylopectin, and the (1 $\rightarrow$ 6)-inter-chain linkages in α -limit dextrins, i.e. they are debranching enzymes. Enzyme preparations from sweet corn also show pullulanase activity (Taylor & Whelan, 1965), but this is distinct from the debranching activity towards amylopectin and phytoglycogen (Manners & Rowe, 1969). We now describe the results of a study of limit dextrinase action on amylopectin and derived α - and β -limit dextrins, and on pullulan.

Results. Two lines of evidence suggested that cereal limit dextrinase preparations would hydrolyse pullulan. First, the minimum specificity requirements of the limit dextrinase of germinated barley (Manners & Rowe, 1968), namely that the enzyme is unable to remove single α -(1 $\rightarrow$ 6)-linked glucose residues from substrates and requires at least one α -(1 $\rightarrow$ 4)-linked glucose residue on both sides of the susceptible α -(1 $\rightarrow$ 6)-glucosidic linkage,

are identical with those described for a bacterial pullulanase by Abdullah *et al.* (1966). Secondly, during the purification of the limit dextrinases from ungerminated oats and germinated barley by continuous electrophoresis or molecular-sieve chromatography, the distributions of limit dextrinase and pullulanase activities were similar. Typical results are shown in Fig. 1. Moreover, when a protein extract from malted sorghum was subjected to molecular-sieve chromatography on Sephadex G-100, the fractions showing hydrolytic activity towards α -maltosyl-(1 $\rightarrow$ 6)-maltotriose also hydrolysed pullulan, and there was no separation of the activities.

During enzyme action on pullulan, paper-chromatographic analysis showed the formation of oligosaccharides with the mobility of hexa- and nona- and higher saccharides, suggesting that random hydrolysis of the substrate had taken place. Evidence that bacterial pullulanase is also an endo-enzyme has been described by Drummond, Smith, Whelan & Tai (1969).

For quantitative enzyme assays, an extract of germinated barley was subjected to continuous electrophoresis as described by Manners & Sparra (1966). Protein fractions were assayed for pullulanase and α -amylase activity, combined as appropriate and freeze-dried. The limit dextrinase preparation was then dialysed for 24 h against 5 mM-sodium citrate buffer, pH 5.2, and assayed at 37°C against various substrates that were 1 mM with respect to the concentration of α -(1 $\rightarrow$ 6)-glucosidic linkages. The rates of hydrolysis, as calculated from the increase in reducing power, which was measured by using a colorimetric method [Nelson (1944) and Somogyi (1952)] and expressed as a percentage of the rate of hydrolysis of α -maltotriosyl-(1 $\rightarrow$ 6)-maltotriose, were as follows: α -maltosyl-(1 $\rightarrow$ 6)-maltotriose, 11; α -maltosyl-Schardinger dextrin, 10; α -maltosyl-(1 $\rightarrow$ 6)-maltotetraose, 103; α -maltotriosyl-(1 $\rightarrow$ 6)-maltotetraose, 204; α -maltotriosyl-(1 $\rightarrow$ 6)- α -maltotriosyl-(1 $\rightarrow$ 6)-maltotriose, 96; pullulan, 84. Enzyme assays using a glucose oxidase method showed that the following substrates were not attacked: maltose, isomaltose, panose, isomaltotetraose, α -glucosyl-(1 $\rightarrow$ 6)-maltotriose and α -glucosyl-Schardinger dextrin. The general trend of the results is similar to that reported for bacterial pullulanase preparations by Abdullah *et al.* (1966) and Walker (1968).

Fig. 1. Distribution of protein and carbohydrases in fractions obtained by the continuous electrophoresis of a protein preparation from ungerminated oats. (a) Distribution of protein (●—●) from measurement of E_{280} , and of α -amylase (○—○), based on the production of reducing sugars from soluble starch, as measured by the Nelson-Somogyi method. (b) Distribution of limit dextrinase (○—○) and pullulanase (●—●) activities, based on the production of reducing sugars from α -limit dextrins and pullulan respectively. (c) Effect of the fractions on the iodine staining power of amylopectin β -dextrin, as measured at 580nm. The electrophoresis was carried out in 5mm-sodium citrate buffer, pH6.0, at 600V, with a Beckman-Spinco model CP cell as described by Manners & Sparra (1966).

When a 1% solution of α -maltosyl-(1→6)-maltotriose was incubated with the barley limit dextrinase, hydrolysis was accompanied by the formation of other oligosaccharides, with the chromatographic mobility of a tetra- and a heptasaccharide. The former was hydrolysed by pullulanase from *A. aerogenes* to give maltose as the sole product, and is therefore characterized as α -maltosyl-(1→6)-maltose. The action of the bacterial pullulanase on the heptasaccharide gave maltose as the major product and maltotriose as the minor

product. They were presumably formed by the transfer of a (1→6)-linked α -maltosyl residue to maltose or to the pentasaccharide respectively. This type of activity was observed in extracts of sweet corn by Taylor & Whelan (1965), who suggested that the activity was a manifestation of a branching enzyme involved in the formation of amylopectin or phytoglycogen. Our results indicate that it is one aspect of limit dextrinase activity. Many carbohydrases are, in fact, transferases, and hydrolysis is simply the commonest form of transfer in which water is the acceptor substrate. Moreover, the barley preparation was devoid of branching enzyme activity.

During this work it became evident that amylopectin β -limit dextrin was a substrate for the cereal limit dextrinases although the parent amylopectin was not attacked. Enzyme action caused a small but significant increase in iodine staining power (see Fig. 1), which was accompanied by a small increase in reducing power and the liberation of maltose and maltotriose. The rate of hydrolysis of amylopectin β -limit dextrin is of the same order of magnitude as that of pullulan.

When a barley limit dextrinase preparation was incubated with amylopectin, glycogen or glycogen β -limit dextrin for periods of more than 40h, there was no detectable change in the light-absorption curves of the polysaccharide-iodine complexes as examined in a Unicam SP.800 spectrophotometer. By contrast, a change in the light-absorption curve of the amylopectin β -limit dextrin-iodine complex was apparent after only 5h. There was a progressive increase in the extinction at 550nm and a movement of the $\lambda_{\max}$ towards the longer wavelengths. These changes are typical of those caused by debranching enzymes. It seems clear that the removal of the maltosyl and maltotriosyl side chains from the amylopectin β -limit dextrin facilitates the formation of an iodine complex with the main chains, since the effective length will increase as debranching proceeds. It might be expected that glycogen β -limit dextrin, which also contains maltosyl and maltotriosyl side chains, would also be hydrolysed. In this case the much higher degree of branching (which is, in fact, about twice that of amylopectin β -limit dextrin) may hinder the formation of an active enzyme-substrate complex. It is believed that the inability of the plant amylopectin-debranching enzyme (R-enzyme) to debranch glycogen is due to similar steric factors (Peat, Whelan, Hobson & Thomas, 1954). However, the optimum size of side chains that can be removed from an amylopectin-type polysaccharide has not yet been determined.

The present results therefore show that cereal limit dextrinases can release (1→6)-linked α -maltosyl or α -maltotriosyl units from both oligosaccha-

rides [e.g. α -maltotriosyl-(1 $\rightarrow$ 6)-maltotriose and maltosyl-Schardinger dextrin] and certain polysaccharides (pullulan and amylopectin β -limit dextrin). In this respect they are similar to the bacterial pullulanases; however, they differ significantly from the latter in having no action on amylopectin, glycogen or glycogen β -limit dextrin.

This work forms part of a research programme sponsored by the Brewing Industry Research Foundation, to whom D.Y. is indebted for a scholarship. We are also grateful to the Science Research Council for an equipment grant, and a research studentship (to J.J.M.).

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