Short Communications

The Specificity of Yeast α -(1 $\rightarrow$ 6)-Glucosidases

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The action of certain yeast enzyme preparations on amylopectin and glycogen causes a limited increase (about 20–50%) in iodine staining power and in β -amyolysis limit (Maruo & Kobayashi, 1951; Gunja, Manners & Khin Maung, 1961). This activity is usually attributed to an enzyme named isoamylase, which is present in both brewing and baking strains of *Saccharomyces cerevisiae*, and hydrolyses the outermost α -(1 $\rightarrow$ 6)-glucosidic inter-chain linkages, which attach linear side chains to the α -glucan macromolecules.

This view is supported by the release of maltosaccharides from baker's-yeast glycogen by isoamylase. When glycogen was incubated with an isoamylase preparation for 72 hr. the extinction of the iodine complex, measured at 420 m μ , increased from 0.45 to 0.54. The enzyme was inactivated by heating, and the digest was then concentrated and subjected to gel filtration on Sephadex G-50. The residual polysaccharide (isodextrin) was thereby separated from low-molecular-weight carbohydrates, which were identified and determined by quantitative paper chromatography as a mixture of maltopentaose (16%), maltohexaose (18%), maltoheptaose (25%), malto-octaose (31%) and maltononaose (10%). When a sample of human liver glycogen whose structure resembled a phosphorylase limit dextrin (Manners, 1954) was incubated with isoamylase, a mixture of maltotetraose and lower maltosaccharides was produced, whereas maltose and maltotriose were released from glycogen β -limit dextrin under similar conditions. All these results show that oligosaccharides containing two or more glucose residues are released during isoamylolysis.

Lee, Nielson & Fischer (1967) reported substantial evidence for the presence in yeast of an enzyme similar to mammalian muscle amylo-1,6-glucosidase. This enzyme also caused an increase in the iodine staining power and β -amyolysis limit of glycogen and amylopectin; this arose from a combination of transferase and glucosidase activity, with the release of glucose as the sole low-molecular-weight carbohydrate. Prolonged action on amylopectin produced an insoluble polysaccharide, which gave a blue-purple stain with iodine. These authors

also suggested that 'it is not unlikely that the amylo-1,6-glucosidase/transferase is responsible for the activity attributed to isoamylase'. This suggestion is incompatible with our observations on the liberation of maltosaccharides from glycogen. It should also be noted that the increase in iodine staining power (about 400% at 680 m μ) of the α -glucans observed by Lee *et al.* (1967) is different from that recorded by Gunja *et al.* (1961), where the greatest increases (about 50%) occurred at the wavelengths of maximum absorption of the α -glucan (about 550 m μ for amylopectin and about 440 m μ for glycogen), and there was little or no change at 680 m μ . Moreover, we have never observed the formation of any insoluble polysaccharides giving a blue stain with iodine.

An alternative explanation is that yeast extracts contain both amylo-1,6-glucosidase and isoamylase, and we now report experimental evidence for this view. In these experiments amylo-1,6-glucosidase activity was assayed by measurement of the incorporation of [14 C]glucose into glycogen or maltosaccharides (Hers, 1964; Manners & Stark, 1967), or by the hydrolysis of α -glucosyl Schardinger dextrin (Taylor & Whelan, 1966) or the branched pentasaccharide 6 3 - α - glucosylmaltotetraose (Illingworth & Brown, 1962).

Protein fractions obtained by the acetone fractionation of bicarbonate extracts of baker's yeast (Gunja *et al.* 1961) caused the β -amyolysis limit of *Helix pomatia* glycogen (average chain length 7 glucose residues) to increase from 37 to 46%, and the iodine staining power at 470 m μ to increase by 32%. (This sample of glycogen was used since its structure is very similar to glycogen phosphorylase limit dextrin, which is the optimum substrate for amylo-1,6-glucosidase.) The products of enzyme action were glucose, maltotriose and maltotetraose, with traces of maltose and maltopentaose, and a residual isodextrin with an average chain length of 10 glucose residues. The same protein fractions also slowly hydrolysed α -glucosyl Schardinger dextrin and the pentasaccharide (to give a mixture of glucose and cyclohexa-amylose or maltotetraose respectively) and catalysed the incorporation of [14 C]glucose into both glycogen and

Table 1. *Comparison of the action of pullulanase and isoamylase on glycogen and amylopectin*

The units of activity of pullulanase and isoamylase were equivalent, as assayed by the increase in iodine staining power of glycogen. The experimental methods were those used by Bathgate & Manners (1966). The β -amylolysis limits are given, together with the percentage increases in parentheses.

Substrate	Original	β -Amylolysis limit (%)	
		After pretreatment with pullulanase	After pretreatment with isoamylase
<i>Ascaris lumbricoides</i> glycogen	49	62 (+13%)	73 (+24%)
<i>Mytilus edulis</i> glycogen	46	63 (+17%)	73 (+27%)
Ram liver glycogen	44	59 (+15%)	69 (+25%)
Pig liver glycogen	50	64 (+14%)	73 (+23%)
Waxy sorghum starch	58	84 (+26%)	84 (+26%)
Potato amylopectin	55	85 (+30%)	81 (+26%)

maltohexaose, the latter giving a branched heptasaccharide.

The protein preparation was fractionated by DEAE-Sephadex column chromatography with 0.05M-sodium citrate buffer, pH 6.0, as eluent. Appropriate column fractions were collected and activity towards glycogen was assayed by iodine staining. The active fractions were then incubated with maltose, α -glucosyl Schardinger dextrin and the branched pentasaccharide. These fractions were devoid of maltase or amylo-1,6-glucosidase activity; they were also inactive by the [14 C]glucose incorporation assay. When incubated with *Helix pomatia* glycogen, the major products were now maltotriose and maltotetraose, and only a trace of glucose was released.

These results clearly establish the presence of a mixture of both isoamylase and amylo-1,6-glucosidase in baker's yeast. The former enzyme resembles pullulanase in its ability to hydrolyse the most accessible interchain linkages in glycogen and amylopectin (cf. Abdullah *et al.* 1966). The effects of these two enzymes on the β -amylolysis limits of various glycogens and amylopectins are compared in Table 1. It should be emphasized, however, that isoamylase has no action on pullulan. The presence of amylo-1,6-glucosidase in yeast would imply a phosphorolytic pathway for the degradation of glycogen. Isoamylase may form part of an alternative pathway; this situation is similar to that in mammalian liver and muscle tissues, where there are at least two pathways for the catabolism of glycogen (Rutter & Brosemer, 1961; Whelan, 1964).

Two consequences of the demonstration of amylo-1,6-glucosidase as an impurity in yeast isoamylase preparations may be mentioned. First, the susceptibility or otherwise of interchain linkages in α -glucans to hydrolysis by isoamylase has been used as evidence for the α -(1 $\rightarrow$ 6)-glucosidic type of linkage (Manners, 1962). Although the earliest

isoamylase preparations almost certainly contained amylo-1,6-glucosidase, the present results in no way alter the previous conclusions, since both enzymes are specific α -(1 $\rightarrow$ 6)-glucosidases. Secondly, when using isoamylase preparations that had not been purified by column chromatography, we have observed the presence of small and variable amounts of glucose among the products of isoamylolysis. Though this was originally attributed to a maltase impurity, inactivation of maltase by variation in pH (Gunja *et al.* 1961) or by the use of specific inhibitors (tris or erythritol; Keleman & Whelan, 1966) did not always arrest the liberation of glucose. This may now be ascribed to amylo-1,6-glucosidase activity.

Extracts of yeast also contain additional α -(1 $\rightarrow$ 6)-glucosidases, which hydrolyse α -(1 $\rightarrow$ 6)-glucosidic linkages in isomaltose and in branched oligosaccharide α -limit dextrans, to give a mixture of maltose, maltotriose and other linear maltosaccharides. This latter activity, usually termed 'limit dextrinase', is similar to that of certain cereal enzymes (MacWilliam & Harris, 1959; Greig, 1963), but is quite distinct from isoamylase and amylo-1,6-glucosidase. For example, the optimum temperature of isoamylase is about 25° (Gunja *et al.* 1961) whereas yeast limit dextrinase is most active at 43°. Moreover, the preparation of isoamylase free of limit dextrinase activity has been described by Kjolberg & Manners (1963).

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