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# Barley Malt α-Glucosidase

## V. Degradation of Starch and Dextrins

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A partially purified α-glucosidase from barley malt has been shown to degrade starch and dextrins to glucose by an end-wise attack.

An α-glucosidase preparation from barley malt has been shown to hydrolyse maltose, isomaltose, and panose.[1,2] Further examination of this glucosidase preparation has given strong evidence of its ability to hydrolyse starch by an end-wise attack yielding glucose as the only reducing sugar. Heat inactivation experiments suggest that the starch degradation (exo α-glucosidase activity) and the α-glucosidase activity are caused by the same enzyme.

The enzyme preparation also catalyses glucosyl group transfer from starch to glucose, when acting on mixtures of starch and glucose in high concentrations.

## MATERIALS AND METHODS

*Enzyme.* The enzyme extracted from high-diastase malt and purified as described previously [1] shows some α- and β-amylase activity when acting on starch. These contaminants can be inactivated by acid and iodine treatment.[3,4] The following procedure was found convenient. The enzyme solution (50 ml) is diluted with water (75 ml), brought to pH 3.1 by addition of 0.5 N acetic acid (about 50 ml) and kept at this pH for 3 h at 25°C. After this period 0.5 N $NH_3$ is added to pH 8.3. The enzyme solution is cooled to 0°C, and 3 ml of 0.005 N $I_2$ in 0.015 M KI is added per 10 ml solution. After 30 min an equivalent amount of 0.005 N thiosulphate is added, and a small precipitate is collected by centrifugation and discarded. pH is adjusted to 5.2 with 0.5 N acetic acid. The precipitate is collected by centrifugation and dissolved to 25 ml at pH 8.3 (adjusted with 0.1 N $NH_3$).

When tested on maltose about 50 % of the α-glucosidase activity is lost by the acid treatment and about 8 % by the iodine treatment. The recovery of activity is dependent on the age of the enzyme preparation; freshly prepared enzyme gave an insoluble precipitate by the acid treatment.

*Enzyme activity determination.* The release of glucose from maltose, isomaltose, and starch (Lintner soluble starch, Merck) was taken as a measure of the maltase, isomaltase, and exo glucosidase activity, respectively. Enzyme solution suitably diluted (200 μl)

is added at time zero to a test tube containing 3.3 mM maltose, 3.3 mM isomaltose, or 0.66 % starch (300 μl) in 0.13 M acetate buffer pH 4.62. To another test tube containing 0.13 M acetate buffer (300 μl) enzyme solution (200 μl) is added for the blank determination. After incubation for 2 h at 37°C the released glucose is determined by addition of 3 ml tris-glucose-oxidase reagent as described by Dahlqvist.[1,5] (Tris buffer inhibits the enzyme reaction). The degree of hydrolysis of the substrate was apparently proportional to the amount of enzyme when the release of glucose was less than 20 μg/0.5 ml reaction mixture. The *enzyme unit* (mU) is the same as that used previously.[1]

*Paper chromatography.* The methods used were essentially as previously described.[6] The $AgNO_3$-NaOH location reagent was used.[7] When necessary the enzyme digests were desalted with Bio-Deminrolit carbonated with $CO_2$ gas.

## RESULTS AND DISCUSSION

For the following reasons the enzyme preparation seems to be reasonably free from α- and β-amylase: Glucose is the only sugar released from starch even at high degrees of hydrolysis as shown by paper chromatography. When the hydrolysis of starch is estimated with the tris-glucose-oxidase reagent selective for glucose and with the non-specific reductometric reagent described by Park and Johnson [8] the same degree of hydrolysis is found by both methods (Table 1). This shows that no fragments of starch accumulate during the degradation. If the glucose released in a starch reaction mixture were released not directly from starch but from maltose (perhaps released from the starch by α- and β-amylase action), the initial steady state concentration of maltose in a given starch reaction mixture could be determined. In an experiment a 0.25 mM maltose solution and a 4.8 % solution of soluble starch were incubated with the same amount of enzyme (0.2 mU/ml) for 2 h. In both reaction mixtures the release of glucose was found to be *ca.* 8 μg of glucose per 0.5 ml reaction mixture. If the glucose released in the starch reaction mixture originated from maltose and not directly from starch the maltose concentration in the starch reaction mixture should be about 0.25 mM or 43 μg of maltose per 0.5 ml. Under conditions where less than 43 μg maltose per 0.5 ml could easily have been detected, a chromatographic examination of the starch reaction mixture showed glucose only. When tris (2-amino-2-hydroxymethylpropane-1,3-diol) at a final concentration of 0.6 M was added to starch

*Table 1.* The release of glucose from starch (0.35 %) by an α-glucosidase preparation from barley malt. Glucose was determined by a specific glucose reagent (I) and by a non-specific reductometric reagent (II).

| Degradation of starch % | (I) mg/ml | (II) mg/ml | Iodine colour $\lambda_{max}$ mμ |
|---|---|---|---|
| 1 | 0.045 | 0.046 | 580 |
| 14 | 0.529 | 0.523 | — |
| 17 | 0.680 | 0.680 | — |
| 21 | 0.801 | 0.823 | — |
| 24 | 0.949 | 0.949 | — |
| 30 | 1.18 | 1.18 | 575 |
| 41 | 1.62 | 1.60 | — |

reaction mixtures (enzyme 2.5 mU/ml, starch 0.4 %, pH 4.6) by which the α-glucosidase activity is totally inhibited,[2] there was no accumulation of maltose, maltotriose or higher oligosaccharides as shown by paper chromatographic examination. α- and β-Amylase are not inhibited by tris under these conditions. The α-glucosidase activity was inactivated by heat (55°C, 20 min) and incubation of the heat inactivated enzyme on starch showed no accumulation of maltose, maltotriose or higher oligosaccharides as appeared from paper chromatographic analysis.

Heat inactivation experiments performed as previously described [2] showed that the maltase and isomaltase activities were inactivated to nearly the same extent as the exo glucosidase activity, suggesting that at least the main part of these activities are caused by the same enzyme (Table 2).

Maltotetraose and maltopentaose (0.4 %) were incubated with enzyme (2.5 mU/ml), and the reaction mixtures were examined by paper chromatography. At the beginning of the reaction maltotetraose gave maltotriose and glucose, and maltopentaose gave maltotetraose and glucose. Later on more glucose was released and, successively, the next lower maltodextrin appeared whereas the higher maltodextrin disappeared. This is in accordance with an end-wise multichain mechanism. After longer reaction times the maltodextrins are totally hydrolysed to glucose. Under the same conditions no degradation of α- and β-Schardinger dextrins (a generous gift from Dr. M. Kelemen) was detected in agreement with the end-wise attack by the enzyme. The degradation of *B. subtilis* α-limit dextrins by the enzyme agreed with an end-wise hydrolysis in which glucose was released. After longer reaction times the α-limit dextrins were totally hydrolysed to glucose. The characteristic blue iodine colour of iodine stained aliquots of starch digests persisted through more than 40 % conversion of the starch to glucose (Table 2), in accordance with an end-wise attack.

The ability of the enzyme preparation to hydrolyse dextran (Dextran 10, Pharmacia) and β-limit dextrin, releasing glucose as the only sugar, and the

*Table 2.* Heat inactivation experiments. (I) Enzyme inactivated at different temperatures for 10 min at pH 8.3. (II) Enzyme inactivated for different periods at 40°C at pH 8.3.

| | | Maltase activity % | Isomaltase activity % | Exo glucosidase activity % |
|---|---|---|---|---|
| I | — | 100 | 100 | 100 |
| | 40°C | 89 | 91 | 89 |
| | 45°C | 64 | 67 | 66 |
| | 50°C | 24 | 28 | 31 |
| | 55°C | 6 | 5 | 9 |
| II | 0 min | 100 | 100 | 100 |
| | 20 min | 86 | 88 | 88 |
| | 40 min | 74 | 78 | 78 |
| | 60 min | 65 | 69 | 73 |
| | 90 min | 56 | 60 | 62 |

ability of the enzyme to hydrolyse isomaltose and panose suggest that the enzyme preparation is able to hydrolyse the $\alpha$-1,6-linkages in starch. When nigeran (a generous gift from Dr. D. J. Manners) was incubated with enzyme a very slight release of glucose could be detected by paper chromatography.

Amylopectin is found to be degraded at a higher initial rate than amylose when examined under identical conditions on a weight basis. Maltose (2 mM), isomaltose (2 mM), and Lintner soluble starch (0.4 %) are hydrolysed at relative initial rates of about 100:10:2, respectively.

The ability of the $\alpha$-glucosidase to catalyse trans-$\alpha$-glucosylation when incubated on maltose in high concentration has been reported.[6] When mixtures of starch and glucose in a concentration of 5 % and 2 %, respectively, were incubated with enzyme (4 mU/ml) for 24 h at 37°C, paper chromatographic analysis of the reaction mixture showed the formation of small amounts of sugars with $R_F$-values corresponding to maltose and isomaltose. The formation of these sugars (especially isomaltose) suggests that the enzyme preparation can catalyse the transfer of glucosyl groups from starch to glucose. When glucose alone, or starch in lower concentrations was acted on by the enzyme no formation of maltose or isomaltose could be detected.

A detailed examination of the enzyme specificity must await further purified enzyme preparations, but the present results show that the $\alpha$-glucosidase preparation from barley malt is able to degrade starch to glucose by an endwise attack. In *e.g.* equine serum and *Candida* yeast similar maltases which can hydrolyse starch to glucose have been found.[9,10]

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