Barley Malt α-Glucosidase

VI. Localization and Development during Barley Germination

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It has previously been shown that barley malt contains an α-glucosidase which catalyses the hydrolysis of maltose, panose, and isomaltose.[1] Accordingly it was of interest to examine the α-glucosidase activity during the germination of barley. Further the location of the α-glucosidase activity in the barley grain was examined by a histochemical procedure.

Barley and barley malt. The samples of ungerminated and germinated barley and of barley malts were kindly supplied by Kongens Bryghus A/S, Copenhagen. Before germination the barley (Rika) was steeped in water for 25 h. After the germination the malt was kilned for 24 h (12 h at a maximum of 65°C and 12 h at a maximum of 85°C). The Herta malt has previously been described.[2]

Extraction of α-glucosidase activity. Portions (250 corns) of ungerminated barley, germinated barley, and kilned barley malt were treated for 5 min in a MSE-blender with 3 % Na_2SO_4 or with water adjusted to pH 8.5 with 0.1 N NH_3 after the blending. The volumes were adjusted to 50 ml and the extractions were continued by shaking for 1 h at room temperature; the extracts were then filtered through S & S 572 ½ folded filters. The clear filtrates to which 1 % of toluene was added were dialyzed for 72 h in "Visking" dialysis tubing (1 cm inflated diameter) against running tap water. The contents of the tubes were then diluted to a known volume and the α-glucosidase activity was determined. In the following these extracts are referred to as the salt extracts and the pH 8.5 extracts.

Determination of α-glucosidase activity was performed as described previously [1] on maltose, panose, or isomaltose (2 mM) at pH 4.6 as a measure of the maltase, panase, or isomaltase activity, respectively. The glucose released was determined by a tris-glucose-oxidase reagent.[2]

When substrate hydrolysis was kept below 5–6 %, the degree of hydrolysis was apparently proportional to the amount of enzyme used.

Histochemical demonstration of α-glucosidase activity. Ungerminated and germinated barley grains (Rika) were fixed in a mixture of 1 % $CaCl_2$ in 4 % formalin for 24 h at 4°C. With a carbon dioxide freezing microtome the grains were sliced into sections of 25 μ thickness. The post-coupling procedure described by Rutenburg *et al.*[3] was used for the histochemical examination. Sections were incubated in 6-bromo-2-naphthyl α-D-glucopyranoside (Carl Roth, Karlsruhe) 0.1 mg/ml at pH 4.6 and 20°C for 15 h. After the incubation the sections were washed and stained in a fast blue B solution, 2 mg per ml 0.1 % $NaHCO_3$, whereby the 6-bromo-2-naphthol released by the α-glucosidase is converted into a purplish-red to blue azo dye. As controls some sections were heated to 100°C for 1 min before the incubation and staining and other sections were incubated in solutions without substrate before the staining. After the staining and washing the sections were mounted with glycerol-gel.

α-Glucosidase activity in ungerminated and germinated barley. The samples of barley (Rika) were extracted and the α-glucosidase activity was determined as described above. The results are shown in Table 1. In ungerminated barley the panase and isomaltase activity is too low for determination. It is seen that apparently 3 % Na_2SO_4 is a better extraction medium for α-glucosidase activity in ungerminated and germinated barley than extraction at pH 8.5, but with kilned malt the pH 8.5 extraction was found to be more effective. The relative amounts of maltase, panase, and isomaltase activity are on an average 100:11:6 for the salt extracts and 100:9:5 for the pH 8.5 extracts. Extracts from Herta malt showed nearly the same relative activities, namely 100:11:6 and 100:10:5, respectively.

The constant ratios of maltase, panase, and isomaltase activity during germination of barley and between different strains of barley malt suggest that only one enzyme is involved in the hydrolysis of the three substrates. Further the ratios found here for the three activities in raw extracts are nearly the same as the ratios found for the more purified α-glucosidase 100:11:6.[1]

During the extraction of barley addition of cysteine (2 mM), $KBrO_3$ (5 mM), or papain (Merck, 2 mg/ml), which often influences the extraction of enzymes from

Table 1. Maltase, panase, and isomaltase activity in salt extracts (3 % Na_2SO_4) and pH 8.5 extracts of ungerminated barley, germinated barley, and kilned malt. The activities are measured as μg of glucose released in 0.5 ml 2 mM substrate in 1 h by suitably diluted extracts. The amount of glucose (μg) released by 1 ml raw extract was now calculated and these figures are given in the table.

		Maltase		Panase		Isomaltase	
		pH 8.5	salt	pH 8.5	salt	pH 8.5	salt
Barley		50	73				
after steeping		75	184				
time of germination	1 day	392	815	35	103	21	62
	2 days	1275	1725	105	183	47	105
	3 days	2205	1748	183	205	89	110
	5 days	2060	1925	212	204	98	103
after kilning		2490	1780	260	190	122	105

cereals, had little or no influence on the amount of extracted α-glucosidase activity.

Localization of the α-glucosidase activity in the grain. In the histochemical examination of the α-glucosidase activity 6-bromo-2-naphthyl α-D-glucoside was used as substrate as described by Rutenburg *et al.*[3] This substrate was shown to be hydrolysed by the more purified α-glucosidase preparation from barley malt. It was found that in ungerminated barley the α-glucosidase activity was located in the embryo and the aleurone layer. The α-glucosidase activity was somewhat lower in the aleurone layer than in the embryo. In barley germinated for 48 h the α-glucosidase activity was greater than in ungerminated barley but located at the same sites. Especially the activity in the scutellum and the scutellar epithelium was much greater as revealed by the deeper blue stain at these sites. Heat inactivated sections or sections incubated without substrate showed a yellow or brown stain at the same sites but this stain could easily be distinguished from the purplish-red to blue stain where the α-glucosidase activity was located. Sections from grains which had not been fixed in formalin showed deeper staining than fixed sections. Experiments by Briggs [4,5] with embryos and endosperms cultured *in vitro* indicate the same location of α-glucosidase activity in barley.

The localization of α-glucosidase activity in distinct areas of the germinated barley grains shows that the α-glucosidase activity in barley malt does not originate from microorganisms developed during the germination of the barley.

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1. Jørgensen, O. B. *Acta Chem. Scand.* **17** (1963) 2471.
2. Jørgensen, B. B. and Jørgensen, O. B. *Acta Chem. Scand.* **17** (1963) 1765.
3. Rutenburg, A. M., Goldbarg, J. A., Rutenburg, S. H. and Lang, R. T. *J. Histochem. Cytochem.* **8** (1960) 268.
4. Briggs, D. E. *J. Inst. Brewing* **68** (1962) 470.
5. Briggs, D. E. *J. Inst. Brewing* **69** (1963) 13.

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