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Barley Malt α-Glucosidase

III. Studies on the Trans-α-glucosylase Activity

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An α-glucosidase isolated from barley malt and purified to some extent shows trans-α-glucosylase activity. Experiments comprising heat inactivation and acid inactivation suggest that the α-glucosidase activity and the trans-α-glucosylase activity are both caused by the same enzyme. Different donor and acceptor substrates were tested and the main oligosaccharides synthesized from maltose were identified as isomaltose, panose, and maltotriose.

The extraction of an α-glucosidase from barley malt and the hydrolytic action of this enzyme preparation have been reported.[1,2] The enzyme preparation shows transglucosylase activity when concentrated maltose solutions are incubated with the enzyme. As several glycosidases show transglycosylase activity [3] it would be of interest to ascertain whether the transglucosylase activity in barley malt is caused by the α-glucosidase or by a trans-α-glucosylase present in the enzyme preparation. The presence of trans-α-glucosylase activity in barley malt extracts has been shown by Pan *et al.*[4] but the activity was not studied further. Anderson and Manners [5] have found trans-β-glucosylase activity in barley.

MATERIALS AND METHODS

Substrates. Isomaltose, maltose, panose, maltotriose, isomaltotriose, and phenyl α-D-glucoside were prepared as described previously.[1,2] D-Glucose was purified by charcoal adsorption. ^{14}C-labelled D-glucose was obtained from the Radiochemical Centre, England. Other reagents used were commercial preparations, analytical-grade purity.

Paper chromatography. Whatman No. 1 filter paper was used. The solvent systems were (I) ethyl acetate-pyridine-water (10:4:3, by vol),[6] (II) butanol-pyridine-water (6:4:3, by vol), (III) water saturated 2-butanone,[7] (IV) butanol-acetic acid-water (4:1:2, by vol),[8] and (V) propanol-ethyl acetate-water (7:1:2, by vol).[9] Descending chromatography was used with solvent systems I, IV, and V and ascending chromatography with solvent

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systems II and III. Location reagents were: (A) silver nitrate-sodium hydroxide,[10] (B) aniline-diphenylamine,[11] (C) naphthoresorcinol,[12] and (D) periodate-benzidine.[13] Flavazole derivatives were located by UV-light.

Preparative chromatography was performed on Whatman No. 31 ET paper using solvent I.

If necessary, enzyme digests were desalted with Bio-Deminrolit (Permutit Co. Ltd., carbonated with CO_2 gas) before the paper chromatography.

1-Phenyl-flavazole derivatives. The 1-phenyl-flavazole derivatives of authentic sugars and of synthesized oligosaccharides were prepared as by Nordin and French [7] and purified by paper chromatography using solvent II. The partial hydrolysis was performed in 0.1 N H_2SO_4 to a degree of hydrolysis of about 0.3. Periodate oxidation of flavazole derivatives was performed as by French *et al.*[14] 1-Phenyl-flavazole-3-aldehyde (m.p. 143—144°C after recrystallisation from acetic acid, reported [14] m.p. 144°C) was prepared from glucose flavazole by periodate oxidation.

Identification of isomaltose flavazole and maltose flavazole. In several tested solvents the difference between the R_F-values of isomaltose flavazole and maltose flavazole is so small that it is difficult to decide conclusively to which of the two flavazoles a given spot corresponds. However, isomaltose flavazole but not maltose flavazole will give 1-phenyl-flavazole-3-aldehyde by periodate oxidation. It was found that this could be tested by two-dimensional paper chromatography. The flavazoles were chromatographed in one direction with solvent II, the spots were sprayed with 1 % sodium metaperiodate, and the paper was allowed to dry. Then the chromatogram was developed in the other direction using benzene-ethanol-water (100:21:2, by vol). The R_F-value of 1-phenyl-flavazole-3-aldehyde was 0.94 in this solvent and other oxidation products scarcely migrated from the origin. In this way 1-phenyl-flavazoles containing 1,6-linkages to the reducing sugar residue can be distinguished from those containing 1,4-(1,5-) linkages.

Enzyme. The enzyme was extracted from high-diastase malt and fractionated as described previously.[1]

Determination of α-glucosidase activity was performed as described previously, using maltose (2 mM) as substrate,[2] and determining the glucose released by means of a tris-glucose-oxidase reagent.[1]

Determination of trans-α-glucosylase activity. The amount of oligosaccharides synthesized from maltose under specified conditions was taken as a measure of the transglucosylase activity. To a small test tube containing 200 µl 0.174 M maltose in 0.1 M acetate buffer, pH 4.1, 50 µl of enzyme was added at time zero. The tube was stoppered and incubated for 20 h at 37°C. (Maltose concentration 0.139 M in the reaction mixture). After the incubation the reaction was stopped by heating for 1 min in a boiling water-bath. Two spots, 20 µl each, of the reaction mixture were applied to a paper for chromatography (one as a locator) and a third spot, 20 µl, of a 0.139 M maltose solution was applied as a blank to the same chromatogram. After developing for 22 h using solvent I the chromatogram was dried and the zones containing oligosaccharides migrating more slowly than maltose were bracketed using the locator strip and cut out. The corresponding area from the blank spot was likewise cut out. The excised sections were cut into smaller segments and eluted with 7 ml water by shaking for 30 min. Using the method of Park and Johnson [15] the sugar contents were determined in 2 ml aliquots of the extracts. As this method is not sensitive to cellulose lint, it was not necessary to filter the extract. Enzyme concentration and extinction (extinction of analysis − extinction of blank) showed a linear relationship when the reducing power of the oligosaccharides in the 2 ml aliquots was lower than the reducing power of 12 µg maltose.

In all transglucosylase experiments 1 % toluene was added.

Enzyme unit. The enzyme unit, U, is the one previously defined [1] using isomaltose (2 mM) as substrate. 1 U = 1000 mU. If maltose (2 mM) is used as substrate instead of isomaltose, the U-value may be found by dividing by 16 as shown previously.[2]

RESULTS AND DISCUSSION

pH-Optimum of transglucosylase activity. The pH-optimum was measured using the conditions given above, except that acetate buffers of different pH-values were used. Setting the activity at pH 4.0 arbitrarily as 100 %, the acti-

vity at pH 3.6, 4.0, 4.6, 5.0, and 5.6 was 90, 100, 93, 90, and 79 %, respectively, giving a pH-optimum very nearly pH 4.0.

This value is apparently lower than the pH-optimum 4.6 given for the α-glucosidase activity,[1] but the pH-optimum for the glucosidase activity falls to a lower pH-value when the substrate concentration is increased.

The influence of heat inactivation on transglucosylase activity and α-glucosidase activity. The enzyme preparation (pH 7.5) was heated for 10 min in a water-bath of the required temperature and then cooled in an ice-bath. The remaining transglucosylase and α-glucosidase activity was determined (Fig. 1). In another heat inactivation experiment the enzyme was kept at 40°C and after different times samples of the enzyme were withdrawn and cooled in an ice-bath, and the remaining enzyme activities were determined (Fig. 2).

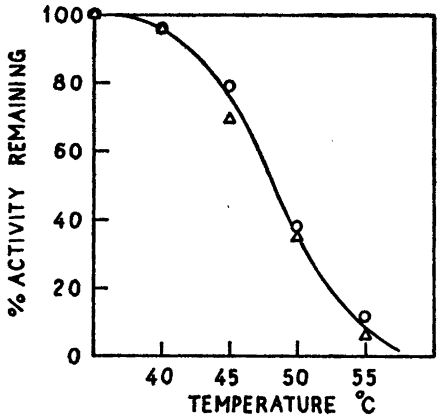

Fig. 1. Heat inactivation of α-glucosidase and trans-α-glucosylase activity from barley malt at different temperatures. Heating time 10 min, pH 7.5. —O— α-glucosidase activity; —△— trans-α-glucosylase activity.

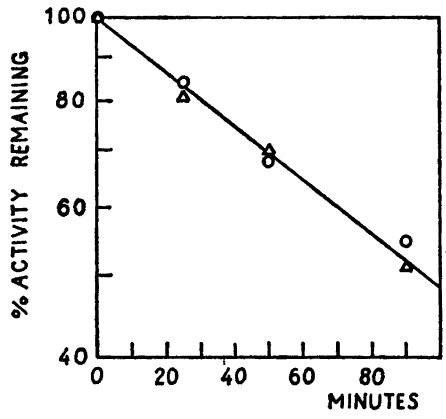

Fig. 2. Heat inactivation of α-glucosidase and trans-α-glucosylase activity from barley malt using different heating times at 40°C and pH 7.5. —O— α-glucosidase activity; —△—trans-α-glucosylase activity.

Acid inactivation. When the enzyme was adjusted to pH 2.7 with 0.1 N HCl at 25°C and kept at this pH for a few minutes a 30 % decrease in the transglucosylase activity as well as the α-glucosidase activity was observed.

All the inactivation experiments show that transglucosylation and glucosidase activity are reduced to the same extent. This suggests that the two activities are caused by the same enzyme.

Transglucosylation. When D-glucose, 0.14 M, was incubated with enzyme (2 mU/ml) at pH 4.1 and 37°C for 20 h no synthetic action could be detected by paper chromatography using location reagent A. No transglucosylation could be detected when maltose solutions of low concentration (2 mM) were incubated using the same experimental conditions. With higher maltose concentrations (0.03—0.3 M) there was a clear indication of transglucosylase action. When maltose solutions, 0.14 M, were incubated for different periods, 24—96 h,

as judged from the R_G-values the same transglucosylation products were formed and there was no significant change in the intensity of the spots.

Donor specificity. Maltose, isomaltose, maltotriose, isomaltotriose, panose, or phenyl α-D-glucoside showed transglucosylase products when 0.14 M solutions of the different sugars were incubated with enzyme (2 mU/ml) at pH 4.1 and 37°C for 20 h, but no transglucosylation could be observed when sucrose, or methyl α-D-glucoside were incubated under the same conditions. (Solvent systems I and II, location reagents A and B).

Tests with maltose and isomaltose as substrates for transglucosylase action, showed maltose to be the more effective substrate, yielding about three times more synthesized oligosaccharides than isomaltose. The amount of synthesized oligosaccharides was measured using maltose as a standard and with the same experimental conditions as under "Determination of trans-α-glucosylase activity". The enzymatic hydrolysis of maltose and isomaltose with the same enzyme preparation shows that maltose is hydrolysed at a rate of about four times that of isomaltose (V = 3.9 and 0.9 μmole glucose/h × ml × mU).[2] This also makes it probable that one enzyme is responsible for both the transglucosylation and the glucosidase activity, even if the ratios are not exactly the same in both cases. This may be due to the different reducing powers of the oligosaccharides synthesized.

Acceptor specificity. When D-glucose, D-mannose, D-galactose, D-fructose, or D-xylose (0.14 M) were mixed separately with maltose (0.14 M) and incubated with enzyme (2 mU/ml) at pH 4.1 and 37°C for 20 h, D-glucose, D-galactose, and D-xylose showed a weak acceptor activity when examined by paper chromatography. D-Mannose and D-fructose showed no acceptor ability. (Solvent systems I and II, location reagents A, B, and, for the detection of transfer products containing fructose, reagent C).

Incubating methanol, isopropyl alcohol, *tert.* butyl alcohol, or phenol separately at a concentration of 0.14 M with maltose and enzyme as above, no transfer products corresponding to the expected α-glucosides could be detected by paper chromatography. (Solvent systems I and IV, location reagents A and D).

Table 1. Chromatographic mobilities relative to glucose (R_G) and maltose (R_M) of the examined sugars.

	R_G Solvent I	R_G Solvent II	R_M Solvent V
Glucose	1.00	1.00	
Maltose	0.60	0.75	1.00
Maltotriose	0.36	0.51	
Isomaltose	0.43	0.54	0.82
Panose	0.26	0.41	
Gentiobiose			0.74
O_1	0.43	0.54	0.82
O_2	0.36	0.51	
O_3	0.26	0.41	

Even if this method is not fully conclusive as to whether a given substrate may be an acceptor in transglucosylation reactions, it is evident that the acceptor substrates tested show a weaker, if any, acceptor ability compared with the acceptor ability of maltose.

Structure of oligosaccharides synthesized from maltose by trans-α-glucosylation. Maltose, 0.14 M, was incubated with enzyme 2 mU/ml at pH 4.1 and 37°C for 42 h. The mixture was boiled for 2 min and 9 ml were applied to Whatman No. 31 ET papers. Three oligosaccharides all migrating more slowly than maltose (O_1, O_2, and O_3) were detected on locator strips and the corresponding zones were cut out and eluted with water. After re-chromatography about 0.5 mg O_1, 6 mg O_2, and 5 mg O_3 were recovered (calculated as maltose).

O_1, O_2, and O_3 produced only glucose when diluted solutions of the three sugars were totally hydrolyzed with the enzyme. The R_G-values of O_1, O_2, and O_3 corresponded to the R_G-values of isomaltose, maltotriose, and panose.

Characterization of O_1 as isomaltose (O-α-D-glucopyranosyl(1→6)-D-glucose). O_1 gave a green colour with location reagent B, and the chromatographic mobility suggested that O_1 was a disaccharide. From O_1 a 1-phenyl-flavazole could be prepared, and by periodate oxidation of this flavazole 1-phenyl-flavazole-3-aldehyde was released. As 1-phenyl-flavazoles only can be prepared when the hydroxyl groups at C_2 and C_3 of the reducing sugar residue are unsubstituted and as 1-phenyl-flavazole-3-aldehyde is only released by periodate oxidation when the hydroxyl group at C_4 is unsubstituted, the facts above suggest that O_1 is either isomaltose or gentiobiose. When O_1 was co-chromatographed with authentic isomaltose or gentiobiose in solvent V, O_1 followed isomaltose and not gentiobiose. Co-chromatography of O_1 flavazole and isomaltose flavazole gave only one spot (Solvent systems II and III).

Characterization of O_2 as maltotriose (O-α-D-glucopyranosyl(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucose). O_2 gave a blue colour with location reagent B. From O_2 a 1-phenyl-flavazole could be prepared. This was partially hydrolyzed by acid, and paper chromatographic analysis of the hydrolyzate showed glucose, maltose (faint), maltose flavazole, and glucose flavazole. By periodate oxidation of O_2 flavazole no flavazole-3-aldehyde was released. Co-chromatography of O_2 with authentic maltotriose and of O_2 flavazole with authentic

Table 2. Chromatographic mobilities of 1-phenyl-flavazole derivatives relative to glucose flavazole.

	$R_{G\text{-flavazole}}$ Solvent II	$R_{G\text{-flavazole}}$ Solvent III	Release of 1-phenyl-flavazole-3-aldehyde by periodate oxidation
Glucose flavazole	1.00	1.00	+
Maltose flavazole	0.90	0.65	−
Maltotriose flavazole	0.79	0.50	−
Isomaltose flavazole	0.91	0.65	+
Panose flavazole	0.73	0.45	−
O_1 flavazole	0.91	0.65	+
O_2 flavazole	0.79	0.50	−
O_3 flavazole	0.73	0.45	−

maltotriose flavazole showed in both cases only one spot. (Solvent systems I, II, and III).

Characterization of O_3 as panose (O-α-D-glucopyranosyl-(1 → 6)-O-α-D-glucopyranosyl-(1 → 4)-D-glucose). O_3 gave a blue-black colour with location reagent B. From O_3 a 1-phenyl-flavazole could be prepared and the partial hydrolyzate of this flavazole was examined by paper chromatographic analysis showing glucose, isomaltose, maltose flavazole (faint), and glucose flavazole. Periodate oxidation of O_3 flavazole released no flavazole-3-aldehyde. Co-chromatography of O_3 with authentic panose and of O_3 flavazole with authentic panose flavazole showed in both cases only one spot. (Solvent systems I, II, and III).

From the above results it is seen that the isolated α-glucosidase from barley malt also shows trans-α-glucosylase activity when acting on maltose if the maltose concentration is sufficiently high. No synthetic action could be observed when the enzyme was acting on glucose.

The enzyme transfers the α-glucosyl radical from maltose to hydroxyl groups at C_4 or C_6 of the non-reducing glucose unit in maltose yielding maltotriose and panose. When glucose is acting as acceptor the glucosyl group can be transferred to the hydroxyl group at C_6 giving isomaltose; transfer to C_4 of the glucose molecule giving maltose cannot be detected by the same method.

To examine if there was any transfer to the hydroxyl group at C_4 of the glucose molecule yielding maltose, a reaction mixture containing maltose (0.14 M), ^{14}C-labelled glucose (D-glucose-1-^{14}C 0.005 M) at pH 4.1 was incubated with enzyme (2 mU/ml) at 37°C for 20 hours. After the incubation 2 μl of the reaction mixture was applied to a paper for chromatography and the chromatogram was developed using solvent I. The radioactivities of the products were measured directly on the paper chromatograms with a Geiger-Müller counting assembly. Radioactive spots with chromatographic mobilities corresponding to glucose, maltose, and isomaltose appeared. The glucose, maltose, and isomaltose spots gave 5340, 170, and 80 counts/min, respectively. This experiment suggests that transfer to the hydroxyl group at C_4 of the glucose molecule takes place.

Transfer of a glucosyl group to another glucose molecule giving maltose has been demonstrated by Edelman and Keys,[16] using an enzyme from wheat germ.

Although the yields of synthesized oligosaccharides given above are not fully quantitative the amounts of maltotriose and panose synthesized in a given reaction mixture seem to be of the same order of magnitude, thus the ability of the enzyme to synthesize α-1,4- and α-1,6-linkages seems to be nearly the same.

The presence of this enzyme activity in barley malt may account for the presence of isomaltose, panose, and in part maltotriose in beer wort.[17]

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