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Characterization of Three Different Hog Intestinal Maltases

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Hog intestinal maltase activity was recently separated into three fractions (maltase I—III) with different heat stability and different K_s values. The characterization of these maltases with respect to their optimum pH, transglycosylase activity, and specificity, is now reported.

Generally, *maltase I* showed properties markedly different from those of *maltases II and III*. The two last maltases, on the other side, had very similar properties except their different sensibility to heat.

All three maltases were found to be α-D-*glucopyranosidases* (glucosido-maltases). *Maltase I* hydrolyzed *sucrose* with a rate 1.7 times the rate for maltose hydrolysis. However, it did not hydrolyze *iso*maltose or phenyl α-D-glucopyranoside. *Maltases II and III* hydrolyzed *isomaltose* and *phenyl α-D-glucopyranoside* but not sucrose. All three maltases hydrolyzed *maltosucrose* (α-D-glucopyranosyl(1→4)α-D-glucopyranosyl(1→2) β-D-fructofuranoside).

Hog intestinal maltase activity was recently separated into three fractions by the method of heat inactivation combined with ion exchange chromatography [1,2]. One of these fractions (maltase III) was not inactivated even when heated in 0.01 M phosphate buffer pH 6.0 at 70°C for 1 h. The other two fractions were more heat-labile, and were rapidly inactivated at 60°C. However, a small difference in heat stability was found also between these two fractions (maltases I and II), which was utilized for the purification of the maltase II fraction [2].

The three maltase fractions are all derived from the microsomes of the intestinal mucosa [1]. Each one of them contributes to about one third of the total maltase activity of the mucosa [1].

The three hog intestinal maltases were found to have different K_s values, which supports the theory that they are different enzymes [2]. In the present paper the optimum pH, the transglycosylase property, and the specificity of the three different maltases are recorded, and their characterization as three different enzymes is discussed.

MATERIALS AND METHODS

Hog intestinal maltases

Maltase preparations were the same fractions from mutual displacement chromatography as were used earlier for K_s determinations [2]. In the text these fractions are referred to as *maltase I, II,* and *III* [1,2]. The specific maltase activity (units of maltase per mg of protein [2]) of the preparations used were 175 for maltase I, 100 for maltase II, and 200 for maltase III.

Substrates

Maltose (monohydrate), cryst., was obtained from Merck A. G. (Germany). For maltase determinations the commercial preparation was used without further purification. In paper chromatography, however, it formed some spots which moved more slowly than maltose. For K_s determinations and for transglycosylase activity studies, therefore, maltose was used which had been purified upon carbon-celite columns [3], and which only formed one spot in paper chromatography. *Isomaltose* (6(α-D-glucopyranosyl) D-glucose) was prepared by enzymatic hydrolysis of dextran, as will be described elsewhere [4]. The hydrolysis of maltose and *iso*maltose was determined with acid copper reagent as described earlier for maltose [2].

Sucrose, cryst., was obtained from J. T. Baker Co. and *αα-trehalose (dihydrate)*, chemically pure, from Pfanstiehl Inc. The hydrolysis of these sugars was determined with the 3,5-dinitrosalicylic acid method [3].

Phenyl-α-D-glucopyranoside. The tetraacetate was obtained by condensation of α-pentaacetylglucose (obtained from Nutritional Biochemicals Corp.) and phenol in the presence of $ZnCl_2$ [5,6]. It had $[\alpha]_D^{20}$ +168.0° (7.2 % in $CHCl_3$) and m. p. 113—114°C. Deacetylation was performed as described elsewhere [7], and the product was crystallized from water. Drying at 50°C *in vacuo* over P_2O_5 yielded anhydrous phenyl α-D-glucopyranoside with $[\alpha]_D^{20}$ +186.0° (3 % in water).

The hydrolysis of phenyl α-D-glucopyranoside was measured at 0.1388 M substrate concentration as in the case of the other substrates. The enzyme solution, therefore, was mixed with an equal volume of 0.2776 M phenyl α-D-glucopyranoside solution. Such a concentrated solution, however, crystallizes at room temperature. It was therefore heated in a boiling water-bath until it became clear, and was then placed in a test-tube in a water-bath at 37°C for 3—5 min before it was mixed with the enzyme solution. The amount of glucose produced was determined with the method of Somogyi and Nelson [8,9] after precipitation of the proteins with the Zn-Ba-reagents of Somogyi [10].

Maltosucrose (α-D-glucopyranosyl(1 → 4) α-D-glucopyranosyl(1→2) β-D-fructofuranoside) was prepared as described earlier [3]. The hydrolysis of this sugar, too, was measured with the method of Somogyi and Nelson.

The rate of hydrolysis of all substrates was measured at 37°C at 0.1388 M substrate concentration in the presence of 0.05 M maleate buffer pH 6.5. When the *initial rate of reaction* was measured, the degree of hydrolysis was not allowed to exceed 10 %. One unit of maltase activity during these conditions produces 1 mg of glucose (= 1 % of hydrolysis in 2 ml of reaction mixture) in 1 h. For comparison of the rate of hydrolysis of different substrates the number of molecules of the different substrates that were hydrolyzed per time unit, was calculated. For a trisaccharide, such as maltosucrose, either the number of glycosylic linkages hydrolyzed or the number of molecules totally hydrolyzed may be calculated [11].

Paper chromatography of sugars was performed as described earlier [3].

^{14}C-Labelled glucose was obtained from the Radiochemical Centre (England). The ^{14}C-labelled sugars were localized on paper chromatograms as described earlier [3].

RESULTS AND DISCUSSION

Optimum pH

The pH activity curves for the three different intestinal maltases are seen in Fig. 1. The optimum pH for the *maltase I* activity was 6.5 with a fairly

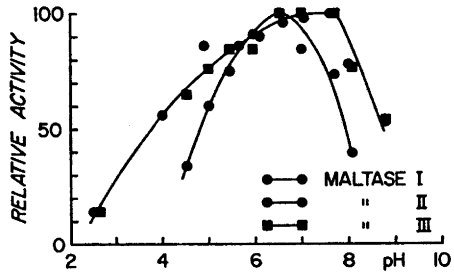

Fig. 1. Influence of pH on the activity of the three different maltases from hog intestinal mucosa. The reaction mixtures contained 5 units of maltase/ml. Buffers: 0.05 M citrate pH 2.5, 0.05 M acetate pH 4.0—5.0, 0.05 M maleate pH 5.5—6.5, 0.025 M veronal pH 7.0—9.0. The pH of the reaction mixtures was measured with a glass electrode.

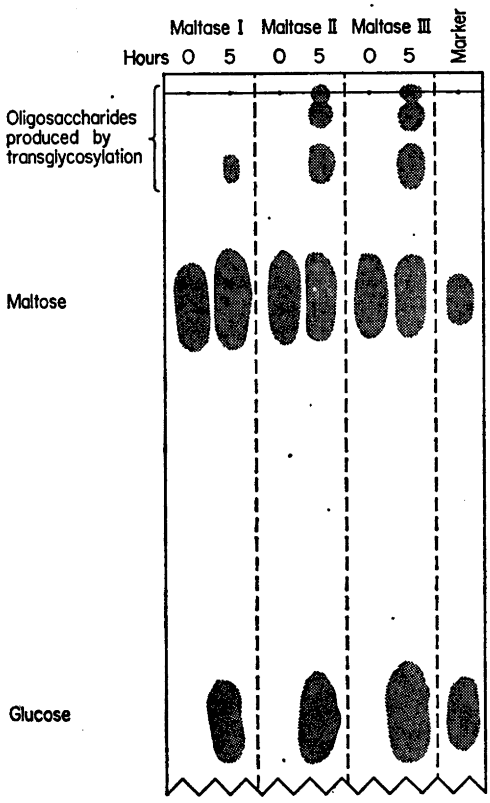

Fig. 2. Formation of oligosaccharides by transglycosylation by the three different hog intestinal maltases. The reaction mixture in all cases contained 50 units of maltase/ml 0.833 M maltose (= 30 % maltose monohydrate), 0.05 M maleate buffer pH 6.5, and a few drops of toluene in each tube. Of the reaction mixtures, 5 µl spots were applied to a paper chromatogram which was run for 60 h. Maltase I produced only one oligosaccharide spot, while maltases II and III produced at least three different oligosaccharide spots.

narrow peak. The shape of this curve coincides with that for the intestinal *invertase* activity [4], which it has not been possible to separate from the maltase I fraction [1,2]. *Maltases II and III* had broader pH activity curves, with optima at pH 6.5—7.5. No difference was found in the shape of the curves for the maltase II and III fractions.

Transglycosylase activity

All three fractions produced slower moving oligosaccharides in a concentrated maltose solution. With *maltase I* only one oligosaccharide spot was found on the paper chromatogram, but with *maltases II and III* a series of at least three different oligosaccharides was formed (Fig. 2).

The transglycosylase activity with ^{14}C-labelled glucose as receptor was also studied. All three maltases incorporated ^{14}C-labelled glucose into the maltose spot (Fig. 3). The rate for this reaction, however, compared with the rate for liberation of glucose, was greater with maltase II and III activities than with maltase I. With maltase I no detectable amount of ^{14}C-labelled glucose was incorporated into the oligosaccharide with slower chromatographic mobility than maltose, but with maltases II and III a great part of the ^{14}C-labelled glucose was incorporated into the oligosaccharides as well (Fig. 3).

The incorporation of ^{14}C-labelled glucose into maltose is *not* caused by the combination of two free glucose molecules. In a 1.65 M (= 30 %) * solution of glucose, under the corresponding experimental conditions, no detectable amount of maltose was formed in 5 h. (When the reaction time was extended to 23 h, however, a faint spot with the same chromatographic mobility as maltose was formed by maltases II and III, but not by maltase I. The reason for the difference is not known. Since the reaction mixture in all cases contained the same number of maltase units per ml, it should have been expected that the reverse reaction (the synthesis of maltose), too, would proceed with the same rate in all three cases. It is well known that the kinetics of the hydrolysis of maltose are such that detectable amounts of maltose are present at equilibrium [12].) In the experiment shown in Fig. 3, therefore, the incorporation of ^{14}C-labelled glucose into maltose (and other oligosaccharides) must be the result of a *transglycosylation*.

Maltases II and III seem to have a stronger transglycosylase activity in relation to their hydrolytic activity than maltase I, since a greater amount of ^{14}C-labelled glucose was incorporated into maltose by maltases II and III than by maltase I under the same experimental conditions. No difference in the transglycosylase activity of maltase II and that of maltase III could be detected.

Rate for the liberation of glucose in concentrated substrate solutions. The amount of glucose liberated (as measured with the acid copper reagent [2]) per time unit by *maltase I* increased with increasing substrate concentration up to more than 5 % (0.1388 M) maltose (monohydrate) (Table 1). With *maltase II and III*, however, the optimal substrate concentration was 1 % (0.0278 M),

* Per cent means gram of solute in 100 ml of solution.

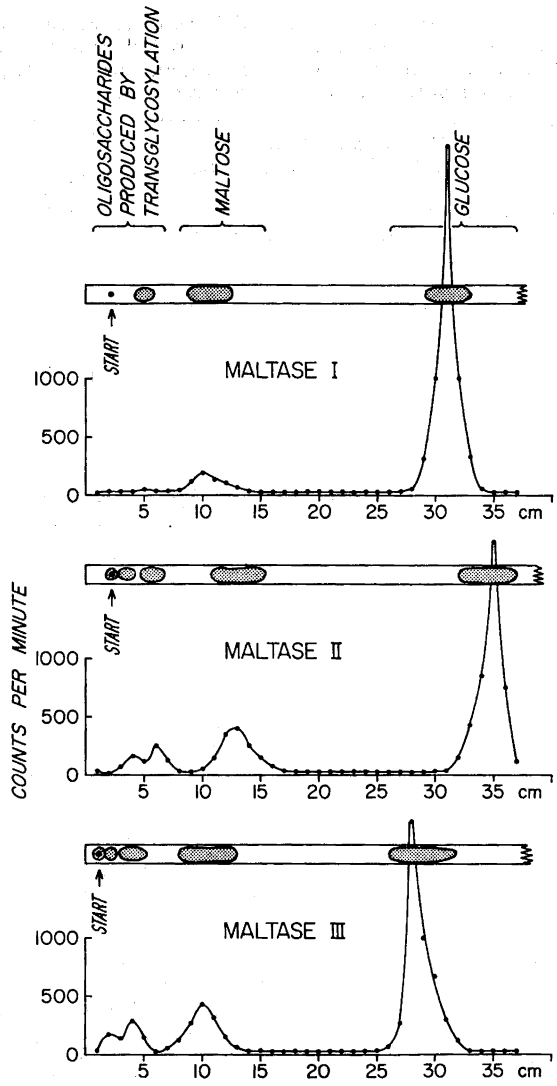

Fig. 3. Transglycosylation by the three different hog intestinal maltases with ^{14}C-glucose as receptor. The reaction mixture contained 50 units of maltase/ml, 0.833 M maltose, 1 mg of ^{14}C-labelled glucose (1 250 000 c.p.m.)/ml, 0.05 M maleate buffer pH 6.5, and one drop of toluene. After 5 h at 37°C the reaction was interrupted by boiling in a water-bath for 2 min, and 5 μl spots of the reaction mixtures were applied to paper chromatograms which were run for 2 days. Each spot contained 6 250 c.p.m. of ^{14}C-labelled sugars. Counting and chemical localization of the sugars were performed on the same paper strip [3]. With *maltase I* 500 c.p.m. (8 % of the total activity) was incorporated into the maltose spot and no detectable amount into the oligosaccharide spot. With *maltase II* 1 250 c.p.m. (20 %) was incorporated into the maltose and 650 c.p.m. (10.4 %) into the other oligosaccharides produced. With *maltase III* 1 250 c.p.m. (20 %) was incorporated into the maltose and 600 c.p.m. (9.6 %) into the other oligosaccharides.

Table 1. The rate of liberation of glucose by three different intestinal maltases in 1 % (0.0278 M) and 5 % (0.1388 M) maltose solutions. The reaction was performed at 37°C in the presence of 0.05 M maleate buffer pH 6.5. The amount of glucose produced was measured with the acid copper reagent.

	Mg of glucose in 1 h per 2 ml of react. mixt.		rate in 1 % maltose / rate in 5 % maltose
	1 % maltose (monohydrate)	5 % maltose (monohydrate)	
Maltase I	6.0	7.5	0.8
Maltase II	6.2	5.3	1.2
Maltase III	11.4	9.5	1.2

and at higher substrate concentration the amount of glucose liberated decreased (Table 1). This effect is possibly caused by the stronger transglycosylase activity of maltases II and III as compared with maltase I.

Specificity of hog intestinal maltases

Sucrose was hydrolyzed by *maltase I* with a rate that was 1.7 times the rate for the hydrolysis of maltose. The invertase and maltase activities of this fraction were not separable by ion exchange chromatography or by heat inactivation, performed in a series of buffers of different pH values [1]. There is strong evidence, therefore, that these two activities are caused by the same enzyme *. *Maltase II* and *maltase III*, on the other hand, did not hydrolyze sucrose. Under the experimental conditions used, a hydrolysis of sucrose with only 1/100 of the rate for maltose hydrolysis would have been detected.

αα-Trehalose (1-(α-D-glucopyranosyl) α-D-glucopyranoside) was *not* hydrolyzed by any of the three maltase fractions. A hydrolysis with only 1/100 of the rate for maltose hydrolysis would have been detected.

Isomaltose (6-(α-D-glucopyranosyl) D-glucose) was *not* hydrolyzed by *maltase I*. A hydrolysis with only 1/200 of the rate for maltose hydrolysis would have been detected. *Maltase II* hydrolyzed iso-maltose with 0.02 times and *maltase III* with 0.05 times the rate for maltose hydrolysis. When heated in the presence of 0.01 M phosphate buffer pH 6.0, the *iso*maltase activity of the maltase II preparation was destroyed below 60°C, while the *iso*maltase activity of the maltase III preparation was not inactivated below 70°C. The inactivation of the *iso*maltase activity of the two preparations ran parallel with that of the maltase activity. It seems probable, therefore, that the *iso*-maltase activity of these two preparations is caused by the same enzyme as the maltase activity.

* Experiments which will be reported in a later paper demonstrate that sucrose and maltose act as competitive inhibitors for each others with this enzyme fraction, which clearly shows that hog intestinal *invertase* is identical with *maltase I*.

Phenyl α-D-glucopyranoside was *not* hydrolyzed by *maltase I*. A hydrolysis with only 1/1 000 of the rate for maltose hydrolysis would have been detected. *Maltase II* hydrolyzed phenyl α-D-glucopyranoside with 0.03, and *maltase III* with 0.05 times the rate for maltose hydrolysis (calculated as the number of molecules hydrolyzed per time unit). As was the case with the *iso*maltase activity of the maltase II and III fractions, phenyl α-D-glucopyranosidase activity had the same sensitivity to heat as had the maltase activity.

Maltosucrose (α-D-glucopyranosyl(1→4)α-D-glucopyranosyl(1→2)β-D-fructofuranoside) was hydrolyzed by all three maltase fractions. By *maltase I* it was hydrolyzed *completely* into glucose and fructose. Calculated as the number of glycosylic linkages hydrolyzed per time unit, the rate for hydrolysis of maltosucrose was 0.8 times the rate for maltose hydrolysis. With the aid of paper chromatography, sucrose was found to be the intermediary product during the hydrolysis of maltosucrose. The trisaccharide is therefore first hydrolyzed into one molecule of glucose and one molecule of sucrose, and then the sucrose molecule is hydrolyzed into glucose and fructose. *Maltase II* and *maltase III* both hydrolyzed maltosucrose into glucose and sucrose, *i.e.* only the *maltose* linkage of the trisaccharide was hydrolyzed. When calculated as the number of glycosylic linkages hydrolyzed per time unit, the rate for hydrolysis of maltosucrose was 0.4 times the rate for maltose hydrolysis for both fractions. The heat inactivation of the maltosucrase activity of these two fractions ran parallel with the inactivation of the maltase activity.

The specificity of *maltase I*, thus, differs markedly from the specificity of *maltase II* and *maltase III*. No difference in specificity was found between the last-mentioned two fractions, however, except that they hydrolyzed *iso*-maltose and phenyl α-D-glucopyranoside at a slightly different rate. All three fractions may be classified as α-D-glucopyranosidases (glucosido-maltases) * since they hydrolyze a number of other α-D-glucopyranosides in addition to maltose.

Possibility of a heat-stabilizing factor

There is no doubt that *maltase I* is an enzyme which differs from the two other fractions. *Maltase II* and *maltase III*, on the other side, are very similar. Except the great difference in their sensitivity to heat [1], the only differences were a small difference in the K_s value for maltose [2], and a slightly different relative rate of hydrolysis of *iso*maltose and phenyl α-D-glucopyranoside. The K_s value as well as the relative rate of hydrolysis of different substrates may be influenced by contaminants in the enzyme preparation, which react with the enzyme. The question therefore arises if the difference in heat sensitivity may be an artifact.

During studies on yeast invertase Myrbäck [14] observed that the course of the heat inactivation did not always follow the kinetics of a first order reaction. The course of the heat inactivation of some preparations should coincide with the existence in them of two different invertases with different

* Leibowitz [13] once postulated the exixtence of *gluco-maltases*, which, in contrast to glucosido-maltases, should have their specificity directed towards the *reducing* component of the maltose molecule. Later workers, however, have not been able to verify the existence of gluco-maltases [12].

heat stability. The K_s value for the invertase activity was not altered, however, during partial heat inactivation, which would have been expected if there really had been two different invertases in the preparation. In order to explain the course of the heat inactivation, therefore, the existence was proposed, not of two different invertases, but of a *heat-stabilizing factor* in a small amount, insufficient to protect the whole enzyme [15].

In order to find out if the maltase III preparation contained a heat-stabilizing factor, an experiment was made for the purpose of showing if the presence of a maltase III preparation, the maltase activity of which had been completely inactivated, could protect maltase II against heat inactivation. A small amount of a *maltase III* preparation, with 216 units/ml of maltase, was heated for 10 min at 100°C. The maltase activity was found to be completely destroyed by this procedure. After cooling, 0.5 ml of the inactivated enzyme preparation was mixed with 0.5 ml of a *maltase II* preparation with 160 units/ml of maltase. When this mixture was heated at 60°C, for 45 min., the maltase activity was found to be completely destroyed. (Before the experiment, both maltase preparations had been dialyzed against 0.01 M phosphate buffer pH 6.0) Thus no heat-stabilizing factor could be demonstrated in the heat-stable maltase preparation.

If maltase II and maltase III are really different enzymes, however, the structural difference between them is probably very small since they are so similar in action and so difficult to separate from each other.

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