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Studies on the Heat Inactivation of Intestinal Invertase, Maltase and Trehalase

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The heat inactivation of the invertase, maltase, and trehalase activities in a solubilized glycosidase preparation from swine intestinal mucosa has been studied.

The trehalase activity was completely separated from the invertase and maltase activities, which is in accordance with earlier findings.

Two different maltase activities were recognized, one *heat-labile* (inactivated at 50°C) and one more *heat-stable* (stable at 70°C when heated for 1 h at pH 6.0). These activities were chromatographically separable, and must therefore be caused by different enzymes.

The invertase activity was inactivated parallel with the heat-labile maltase activity. These two activities are, however, not quite identical, since a heat-labile maltase with low invertase activity was isolated chromatographically. The heat-labile maltase activity is possibly caused by two different enzymes, one of which is combined with the invertase activity, while the other one is not.

Particle-bound glycosidases in microsomes show the same behaviour as the solubilized enzymes when exposed to heat, which demonstrates that all the activities observed have their origin in the intestinal mucosa and that the properties of the enzymes are not altered during solubilization by trypsin digestion.

The hydrolysis of different α-D-glucopyranosides, including sucrose, by intestinal mucosa has been proposed to be caused by one single α-D-glucopyranosidase [1,2]. Recently has, however, been found that intestinal trehalase activity in preparations from swine intestine could be separated from the invertase and maltase activities by anion exchange chromatography [3]. The invertase and maltase activities formed one common peak, but this peak was not homogeneous, since the maltase/invertase activity quotient was lower in the beginning of the peak than in its end. It was therefore apparent, that the intestinal mucosa contains several different enzymes capable of hydrolyzing α-D-glucopyranosides, but for further studies a more complete separation is necessary.

Different enzymes show great variations in their sensitivity to heat inactivation during standardized conditions. This fact may be used for distinguishing

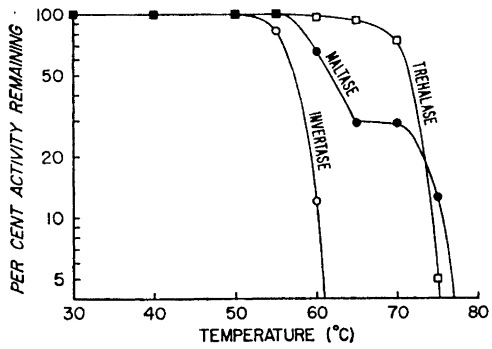

Fig. 1. Heat inactivation of intestinal glycosidases at continuously increasing temperature (gradient 1°C per min). The enzyme solution used was purified by ethanol fractionation and contained 90 U of invertase, 180 U of maltase, 20 U of trehalase and 6.1 mg of protein per ml in 0.01 M phosphate buffer pH 6.0.

different enzymes in mixture. This method has been used for the demonstration of two different bromo-naphthyl-β-D-galactopyranosidase activities in calf intestinal mucosa, only one of which was combined with lactase activity [4]. Invertase from human jejunum is known to be more sensitive to heat than yeast invertase [5], but a comparison between the heat sensitivities of the different α-D-glucopyranosidase activities of intestinal mucosa seems never to have been performed.

In the present paper is described a study of the heat inactivation of the invertase, maltase, and trehalase activities of a solubilized glycosidase preparation from swine intestinal mucosa. The heat inactivation of different fractions from an anionic exchange chromatogram of this preparation also was studied. In addition the heat inactivation of these activities in their non-soluble form in a preparation of microsomes from swine intestinal mucosa was studied.

RESULTS AND DISCUSSION

A. Heat inactivation of solubilized glycosidases

The enzyme preparation used was a solubilized preparation of glycosidases from swine intestinal mucosa, purified by ethyl alcohol fractionation [3]. It contained 90 U of invertase, 180 U of maltase, 20 U of trehalase, and 6.1 mg of protein per ml. Before use the solution was dialyzed against 0.01 M phosphate buffer pH 6.0.

Inactivation at increasing temperature. In a preliminary experiment, 5 ml of the enzyme solution was heated in a waterbath at increasing temperature (increasing 1°C per min.). At intervals 0.5 ml samples were taken, and immediately blown down into test-tubes chilled with crushed ice, in which they were stored until analysed.

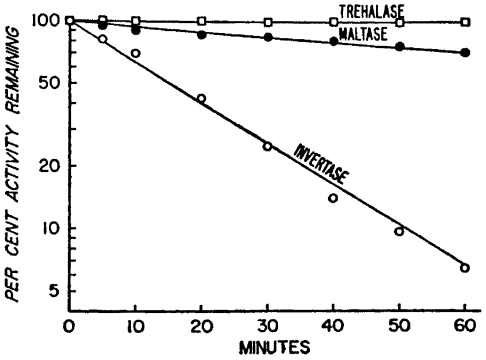

Fig. 2. Heat inactivation of intestinal glycosidases at 50°C. Enzyme solution the same as in Fig. 1.

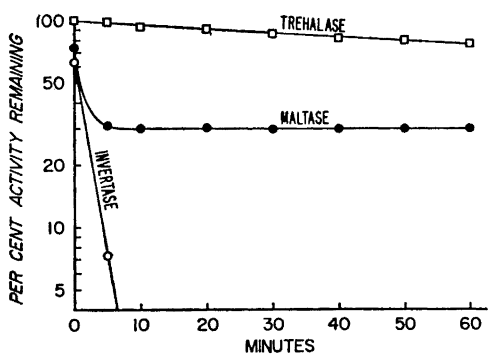

Fig. 3. Heat inactivation of intestinal glycosidases at 60°C. Enzyme solution the same as in Fig. 1.

The different activities investigated were found to differ widely in their sensitivity to heat. The invertase actvity was abolished between 50 and 60°C while the trehalase activity was not influenced below 65°C (Fig. 1). These activities must therefore be caused by two different enzymes, which has also been revealed by ion exchange chromatography [3]. The maltase was inactivated in two steps. 70 % of the maltase activity disappeared in the same temperature interval as the invertase activity, while the remaining 30 % of the total maltase activity were inactivated first when the temperature exceeded 70°C.

Inactivation at constant temperature. At constant temperature the heat inactivation of enzymes usually follows the kinetics of a first order reaction, *i.e.* if the logarithm of the remaining enzymatic activity is plotted against time, a straight line is obtained [6].

To reach the desired temperature within reasonable time, it was necessary to heat the enzyme solution, in a thin-walled test tube, in a preheating bath which has a temperature 8°C higher than the desired inactivation temperature. When the desired temperature was reached (within 45 sec.) the tube was immediately transferred to the inactivation bath, and in the same moment a zero-time sample was taken and a stop-watch was started. At time intervals samples were taken as described above. The glycosidase activities in the samples are expressed as per cent of the original activity (before exposure to heat).

At 50°C (Fig. 2) the invertase activity diminished to 50 % of its original value in 18 min. The maltase was inactivated more slowly, and the trehalase activity was not affected at all.

At 60°C (Fig. 3) 99 % of the invertase activity was destroyed in about 10 min. The maltase activity rapidly diminished to 30 % of its original value, but even after heating for 1 hour at 60°C the maltase activity was not further affected. The trehalase activity decreased only very slightly.

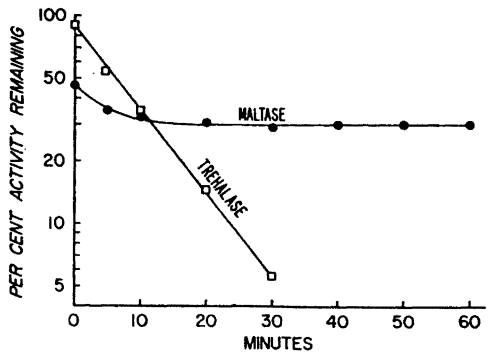

Fig. 4. Heat inactivation of intestinal glycosidases at 70°C. Enzyme solution the same as in Fig. 1.

At 70°C (Fig. 4) the invertase activity was practically immediately destroyed, and the trehalase activity was rapidly diminishing, following first order kinetics. The maltase activity decreased immediately to 30 % of its original value, but was then not measurably affected. At higher temperature also the maltase activity was completely destroyed.

The maltase activity apparently consists of two components, one more *heat-labile*, constituting 70 % of the total activity, and one more *heat-stable*, constituting 30 % of the total maltase activity. The maltase activity must therefore be caused either by two different enzymes or by one enzyme which in inactivated in two steps. As will be seen below, the heat-labile and the heat-stable maltase activities are chromatographically separable, and must therefore be caused by two different enzymes.

From the inactivation experiment at 70°C (Fig. 4) it is clearly seen that the trehalase activity is independent of both the invertase and heat-labile maltase activities (which are practically immediately destroyed) and the heat-stable maltase activity (which is not affected at all). This is in accordence with the earlier reported isolation of a trehalase fraction from intestinal mucosa which had no maltase or invertase action.

The relations between the heat-stable and heat-labile maltase activities and the invertase activity were further investigated by analysis of fractions obtained by chromatography of these activities upon DEAE-cellulose.

B. Heat inactivation of fractions from DEAE-cellulose chromatography

In chromatography upon DEAE-cellulose [3] the invertase and maltase activities form one common peak, which, however, is inhomogeneous. The maltase/invertase activity quotient is 0.6 in the beginning of the peak, while it rises to over 4 in its end.

Chromatography on DEAE-cellulose. A solubilized glycosidase preparation, containing 24 000 U of invertase, 48 000 U of maltase, and 400 mg of protein in 0.05 M phosphate buffer pH 6.0 was applied to a column containing 10 g (dry weight) of DEAE-cellulose. The column was first eluted with 2 liters of 0.05 M phosphate buffer pH 6.0. This fraction contained no maltase or invertase activity and was discarded. The column was then eluted with phosphate buffer of the same pH with step-wise increasing molarity (Table 1).

Table 1. Chromatography of intestinal maltase and invertase on DEAE-cellulose with step-wise elution.

Fraction No.	Molarity of buffer	Volume	Mg of protein	U of invertase	U of maltase
I	0.065	300 ml	24	3 300	2 000
II	0.150	300 »	229	20 000	42 000
III	0.200	200 »	7.6	400	1 700

Concentration of the fractions. After dilution of the fractions with distilled water to give a final concentration of 0.05 M buffer, the fractions were applied to small DEAE-cellulose columns (dry weight of DEAE-cellulose 1 g for fraction I and III and 5 g for fraction II) and thereafter eluted with 50 ml of 0.2 M phosphate buffer pH 6.0. Before heat inactivation the solutions were dialyzed against 0.01 M phosphate buffer pH 6.0.

Heat inactivation of the fractions obtained. Fraction I contained after concentration 80 U of invertase, 50 U of maltase, and 0.6 mg of protein per ml. This means that the maltase/invertase activity quotient is 0.6. When heated at 48°C both the invertase and the maltase activity diminished, following first order kinetics, and closely following each other (Fig. 5). The maltase activity in this fraction is apparently all heat-labile. The question arises if the maltase

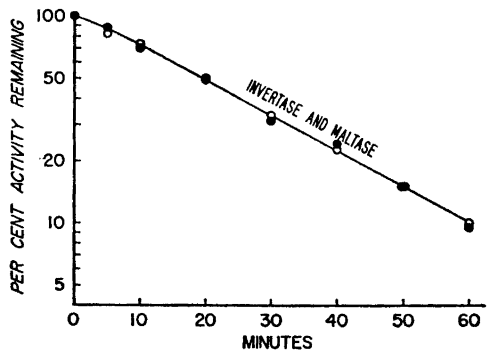

Fig. 5. Heat inactivation at 48°C of fraction I from DEAE-cellulose chromatography. The solution contained 80 U of invertase, 50 U of maltase, and 0.6 mg of protein per ml in 0.01 M phosphate buffer pH 6.0. The maltase activity is all heat-labile, and invertase and maltase are inactivated parallel.

and invertase activities in this preparation are caused by the same enzyme. When a sample of this fraction was rechromatographed on DEAE-cellulose, using gradient elution, the invertase and maltase activities appeared as one peak, which was quite homogeneous. Heat inactivation was performed in buffers of varying pH in order to find any differences in behaviour of the maltase and the invertase activities, but no difference was found (Table 2).

Table 2. Heat inactivation of fraction I at 45°C in varying buffers. Maltase/invertase activity quoient 0.6.

Buffer	Half-time for the invertase activity, min	Half-time for the maltase activity min
0.01 M acetate pH 5.2	10	10
0.01 M phosphate pH 6.0	90	90
0.01 M phosphate pH 7.0	80	80
0.01 M veronal pH 8.0	3	3

Fraction III contained after concentration 10 U of invertase, 41 U of maltase, and 0.2 mg of protein per ml. When the solution was heated at 60°C, the invertase activity rapidly disappeared together with 20 % of the maltase activity (Fig. 6). The remaining 80 % of the maltase activity were however not affected even after heating for 1 h. This part therefore consists of heat-stable maltase. That the heat-labile and the heat-stable maltase activities are chromatographically separable, demonstrates that they are caused by two different enzymes.

It is apparent, that the heat-stable maltase has no invertase action. On the other side the invertase fraction obtained (fraction I) had (heat-labile) maltase activity, with a maltase/invertase activity quotient of 0.6. This maltase activity could not be separated from the invertase activity, and there

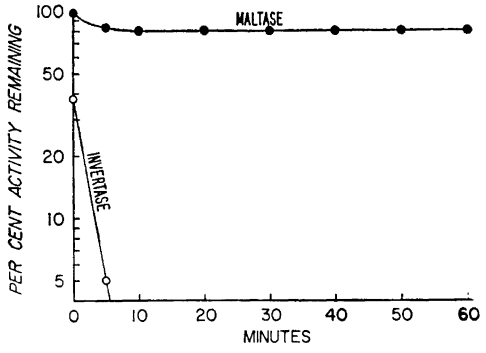

Fig. 6. Heat inactivation at 60°C of fraction III from DEAE-cellulose chromatography. The solution contained 10 U of invertase, 41 U of maltase, and 0.3 mg of protein per ml in 0.01 M phosphate buffer pH 6.0. The maltase activity is mainly heat-stable.

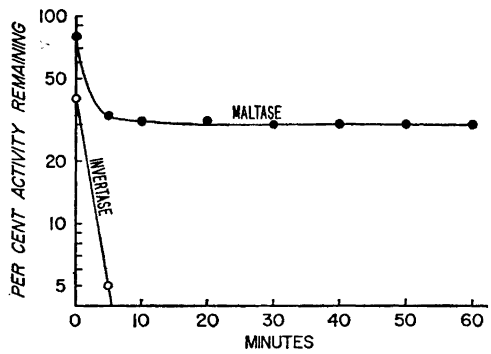

Fig. 7. Heat inactivation at 60°C of a preparation obtained by repeated rechromatography of the middle fraction from DEAE-cellulose chromatography. The solution contained 3.9 U of invertase, and 67.5 U of maltase per ml. In spite of the low invertase activity, the major part of the maltase activity is heat-labile.

are reasons to believe that these two activities are caused by the same enzyme. The original enzyme preparation had, however, a maltase/invertase activity quotient of 2.0. Since the heat-labile maltase activity amounted to 70 % of the total maltase activty, this preparation had a heat-labile maltase/invertase activity quotient of 1.4. It stands therefore clear, that even if the invertase has maltase activity, it cannot cause more than 50 % of the heat-labile maltase activity in the original preparation.

When fraction II, which contained the main part of the original enzyme activity, was rechromatographed on DEAE-cellulose, again a first fraction with a maltase/invertase activity quotient of 0.6, and a last fraction mainly consisting of heat-stable maltase were obtained. By repeated collection and rechromatography of the middle fraction, a preparation was obtained which contained all three activities, but in which the heat-labile maltase/invertase quotient was gradually increased. After four times repeated chromatography, this fraction had a heat-labile maltase/invertase quotient of 12.1 (Fig. 7). There exists therefore one heat-labile maltase activty which has no (or very low) invertase action. On DEAE-cellulose it is only possible to purify this activity partially, wherefore more suitable separation methods are under investigation. The relation between this invertase-free heat-labile maltase activity and the maltase activity which accompanies the invertase activity will be further studied.

If it is supposed that there exist *two* different *heat-labile* maltases, the following intestinal maltases will be recognized:

Maltase I: heat-labile maltase activity accompanying the invertase activity and amounting to 30 % of the total maltase activity.

Maltase II: heat-labile maltase activity without invertase action, amounting to 40 % of the total maltase activity.

Maltase III: heat-stable maltase activity, amounting to 30 % of the total maltase activity.

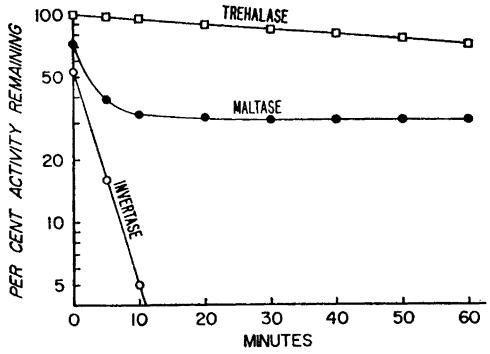

Fig. 8. Heat inactivation at 60°C of particle-bound intestinal glycosidases. The sample contained 35 U of invertase, 70 U of maltase, 10 U of trehalase and 7 mg of protein per ml in 0.01 M phosphate buffer pH 6.0.

C. Heat inactivation of particulate glycosidases

In the method used for preparation of soluble glycosidases, the intestinal contents are not removed from the mucosa [3]. This offers the possibility for contamination with enzymes secreted from other organs or bacterial enzymes. It has, however, been found that the invertase obtained in this way has the same properties as the invertase in washed microsomes from the intestinal mucosa, and that the relation of the maltase and trehalase activities to the invertase activity is unaltered [3]. It must also be considered, that the solubilization by trypsine digestion may alter the properties of the enzymes. This seems, however, not probable, because the enzymatic activity is unaltered [3].

The behaviour of particle-bound intestinal glycosidases during heat inactivation was studied on a desoxycholate-extract from washed intestinal microsomes [3]. This extract is known to contain only particle-bound glycosidases, which can be sedimented in a centrifuge if the desoxycholate is removed [3]. It contained 35 U of invertase, 70 U of maltase, 10 U of trehalase, and 7 mg of protein per ml. The extract was dialyzed against 0.01 M phosphate buffer pH 6.0 before use.

At 60°C (Fig. 8) the invertase activity disappeared within 10 min together with 68 % of the total maltase activity. The remaining 32 % of the maltase activity was not further affected. The trehalase activity was only very slowly diminishing. This behaviour is quite identical with that which was found with solubilized glycosidases (Fig. 3). This shows that all the activities observed have their origin in the microsomes of the intestinal mucosa, and that the properties of the enzymes are not altered during their solubilization by trypsin digestion.

MATERIALS AND METHODS

Enzymes. Solubilized glycosidases were prepared from swine intestinal mucosa by trypsin digestion and ethyl alcohol fractionation as described earlier [3]. *Particle-bound glycosidases* were prepared from washed microsomes from swine intestinal mucosa by extraction with sodium desoxycholate as described earlier [3].

Substrates. Sucrose was obtained from J. T. Baker Co., maltose (monohydrate) from Merck A. G. (Germany), and trehalose (dihydrate) from Pfanstiehl Chemical Co.

Determination of glycosidase activity was performed at 37°C. The reaction mixture had 0.1388 M substrate concentration and contained 0.05 M maleate buffer of pH 6.5 for invertase and maltase and 6.0 for trehalase. The degree of hydrolysis was determined with the 3,5-dinitrosalicylic acid reagent of Sumner [7] for sucrose and trehalose, and with the acid copper reagent of Tauber and Kleiner [8] for maltose. The degree of hydrolysis was not allowed to exceed 10 % in one hour. During these conditions the amount of monosaccharides produced was proportional to the glycosidase activity (zero order reaction). One U of glycosidase activity means the activity producing 1 mg of monosaccharides in one hour during the above conditions.

Protein determination was made with the method of Lowry *et al.*[9] A standard curve was made with human serum albumin (kindly supplied by A. B. Kabi). *Spectrophotometric readings* were made with a Beckman B spectrophotometer.

Diethyl-aminoethyl-cellulose (DEAE-cellulose) was prepared by the method of Peterson and Sober [10]. It contained 0.78 mequiv. titrable hydroxyl groups per g dry weight.

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REFERENCES

1. Weidenhagen, R. *Ergeb. Enzymforsch.* **1** (1932) 168.
2. Neuberg, C. and Mandl, I. in Sumner, J. B. and Myrbäck, K. *The Enzymes* Vol. I. Part 1., New York 1950.
3. Borgström, B. and Dahlqvist, A. *Acta Chem. Scand.* **12** (1958) 1997.
4. Heilskov, N. S. C. *Studies on Animal Lactase,* Dissertation, Copenhagen 1956.
5. von Euler, H. and Myrbäck, K. *Z. physiol. Chem. Hoppe-Seyler* **115** (1921) 68.
6. Moelwyn-Hughes, E. A. in Nord F. F. und Weidenhagen, R. *Handbuch der Enzymolgie,* Leipzig 1940.
7. Hostettler, F., Borel, E. und Deuel, H. *Helv. Chim. Acta* **34** (1951) 2132.
8. Tauber, H. and Kleiner, I. S. *J. Biol. Chem.* **99** (1932) 249.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem.* **193** (1951) 265.
10. Peterson, E. A. and Sober, H. A. *J. Am. Chem. Soc.* **78** (1956) 751.

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