STUDIES ON ACID α-1,4-GLUCOSIDASE FROM BOVINE SPLEEN

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Since the discovery by Hers that tissues obtained from patients with Pompe's disease fail to hydrolyze maltose and the outer chains of glycogen at acid pH values, whereas normal tissues have this activity, a deficiency of an α-1,4-glucosidase with an acidic pH optimum has been implicated to be important in the accumulation of glycogen in these patients (Hers, 1963). Rosenfeld (1964) has reported the same type of enzyme in various animal tissues. He found that the spleen contains the highest concentration of this enzyme. This enzyme forms glucose from maltose as well as from the outer chains of glycogen. It also catalyzes the tranfer of a glucosyl unit from maltose to glycogen.

In this communication we report the purification of this enzyme and some evidence for its existence in multiple forms.

Bovine spleen was homogenized with acetate buffer (pH 4.8) and fractionated successively with solid ammonium sulfate, isopropanol, ethanol and acetone.

The enzyme was further purified by DEAE sephadex A-50 and phosphocellulose column chromatographies and acetone fractionation. All procedures were carried out at 0°-4°C except

TABLE 1

PURIFICATION OF ACID α-1,4-GLUCOSIDASE

Fraction	Volume (ml)	Maltase			Glucoamylase		
		Units	Specific Activity	Recovery %	Units	Specific Activity	Recovery %
1. Crude extract	14840	3.8	0.41	100	4.1	0.44	100
2. 0.33-0.55 $(NH_4)_2SO_4$ Precipitate	1030	42.3	2.39	77.2	45.5	2.57	76.6
3. 0.33-0.60 Isopropanol Precipitate	195	155.0	18.45	53.6	218.3	25.90	69.6
4. 0.20-0.50 Ethanol Precipitate	49	272.9	21.15	23.7	767.0	59.45	61.4
5. 0.40-0.50 1st Acetone Precipitate	12	922.0	104.77	19.8	1871.7	212.69	35.9
6. Phosphocellulose Fraction	24	383.6	255.73	16.3	579.3	386.20	22.7
7. DEAE-Sephadex A-50 Fraction	17	522.2	652.75	15.7	679.0	848.74	18.8
8. 0.10-0.50 2nd Acetone Precipitate	10	840.1	763.63	14.8	1092.0	992.72	16.2
9. 1st Crystallization	4	1263.4	971.84	8.9	1334.0	1026.15	8.7
10. 2nd Crystallization	4	1260.5	969.61	8.9	1348.7	1037.46	8.7

Units are expressed as micromoles of hydrolyzed glucosidic bonds per 30 minutes.

for the isopropanol, ethanol and acetone fractionations which were done at -20°C. The purification procedure is summarized in TABLE 1. The final enzyme preparation in 0.05M acetate buffer, pH 4.8, could be crystallized by addition of acetone. Crystals appeared when the concentration of acetone reached 5% v/v. To complete the crystallization further acetone was added slowly over a period of 4 hours to give a final concentration of 20% v/v at -20°C. The enzyme crystallized as large thin, fragile looking plates (Fig 1), and was purified about 2400 fold over the crude extract.

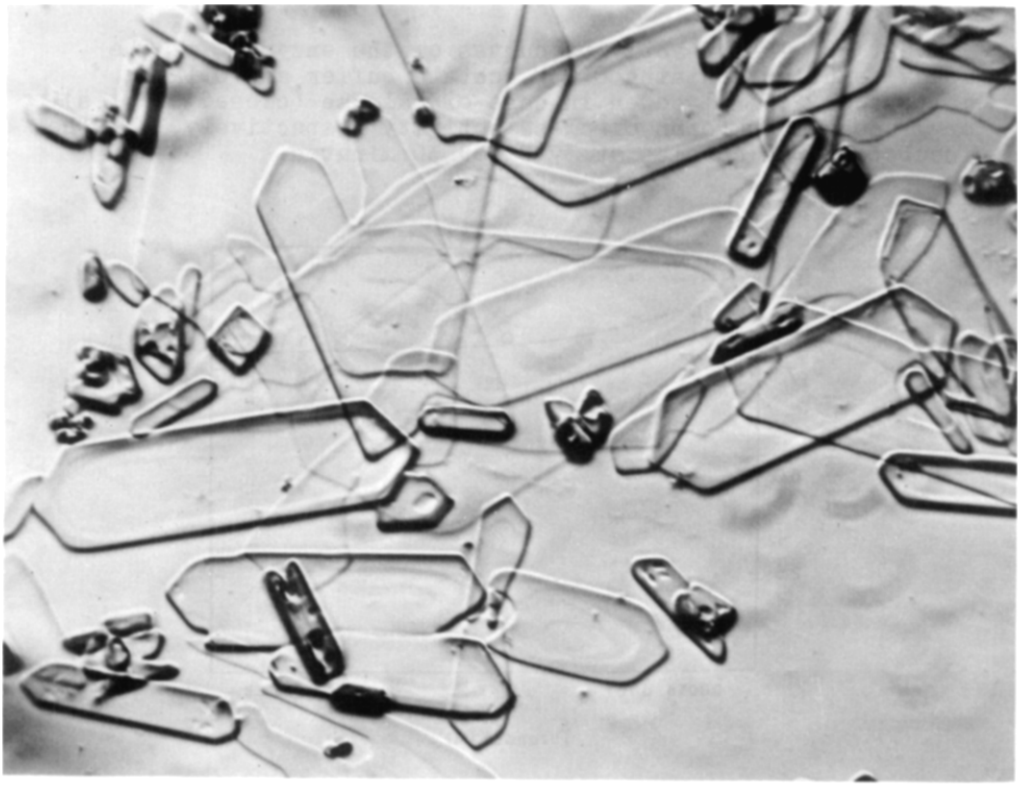

Fig. 1. Crystals of the acid α-1,4-glucosidase (X 150).

Two assay methods were employed in the experiments. In the one method, glucose liberated from maltose was measured

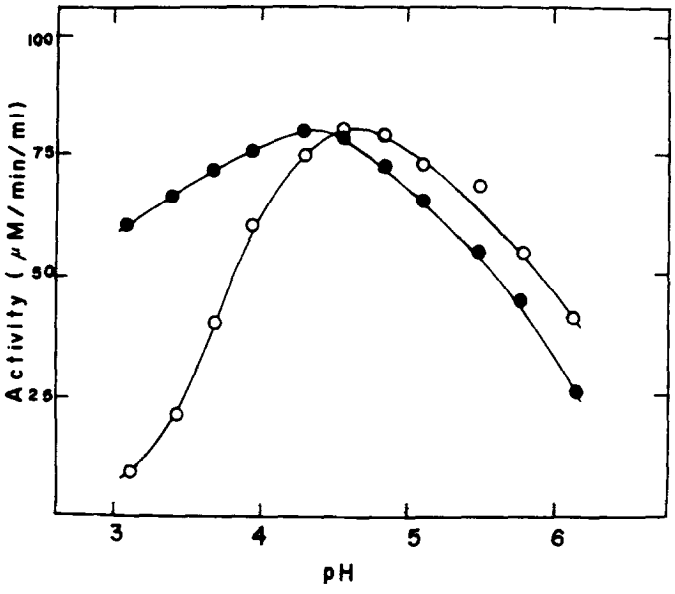

Fig. 2. The pH-activity curves of the enzyme. The assay solution contains 0.05M acetate buffer and 0.125M maltose or 2.5% glycogen in case of maltase (closed circles) or glucoamylase (open circles) activity respectively. The incubation was carried at 37°C for 30 minutes.

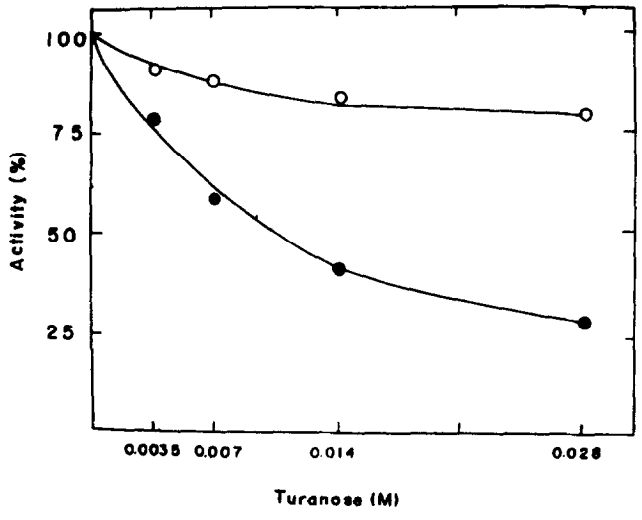

Fig. 3. Inhibition by turanose. Experiments were carried out at 37°C and pH 4.8.
Maltase (closed circles). Glucoamylase (open circles).

after 30 minutes incubation at 37°C in acetate buffer pH 4.8. The activity measured is defined as maltase activity. In the other method, glycogen was used as substrate instead of maltose. The activity measured is defined as glucoamylase activity. The glucose liberated was measured by a glucose oxidase method (A. Dahlqvist, 1964) with some modifications.

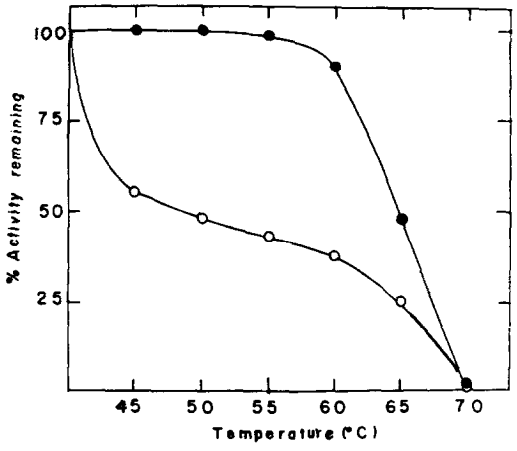

Fig. 4. Heat sensitivities of maltase activity (closed circles) and glucoamylase activity (open circles). Activity was assayed after treatment at the indicated temperature for 15 minutes.

Specific activity was expressed as units per mg protein where protein was determined by Lowry's method, (1951) using bovine albumin as a standard.

As shown in TABLE 1, the ratio of maltase activity to glucoamylase activity differed at each purification step. The two activities were affected differently by pH as shown in Fig 2. Turanose, an inhibitor of acid α-1,4-glucosidase, (Hers, 1963) inhibited the activity of both maltase and glucoamylase but the two activities were affected differently by increasing concentrations of turanose, as shown in Fig 3. In addition, the heat sensitivities of the two activities differed, as shown

in Fig 4. These results suggest that the acid α-1,4-glucosidase exists in two or more different forms which differ in specificity towards maltose and glycogen.

Experiments are in progress to identify these components.

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