ACID α-D-GLUCOSIDASES FROM PLANT SOURCES

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SUMMARY: Evidence is presented for the presence in plant extracts of α-glucosidases with acidic pH optima. This type of enzyme has previously been described only in mammalian sources.

Many mammalian tissues have been shown to contain acid α-glucosidase (α-D-glucoside glucohydrolase, E.C. 3.2.1.20) activity. This enzyme has been purified from bovine liver (1) and spleen (2), rat liver (3, 4), rabbit muscle (5) and human kidney (4). The properties of this enzyme from a number of different mammalian sources have been reviewed (6). It is a lysosomal enzyme which degrades both maltose and glycogen and which plays an important role in the *in vivo* degradation of glycogen. Absence of this enzyme leads to a serious disorder of glycogen metabolism (Type II glycogenosis, Pompe's disease, (7)).

In a previous paper (8) we have mentioned the presence in crude extracts of sweet corn of an α-glucosidase with optimal activity between pH 3.1 and 3.8. As far as we are aware this is the first report of an acid α-glucosidase from a plant source.

We have now purified this enzyme by column chromatography on CM-cellulose, DEAE-Sephadex A-50 and Sephadex G-200. Full details of this purification procedure will be given elsewhere. Passage of the resulting preparation through hydroxylapatite and elution with a gradient of phosphate buffer yielded three

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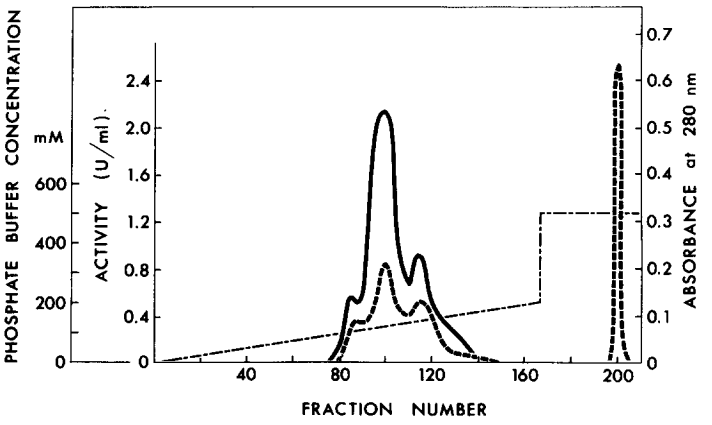

Fig. 1 Fractionation of purified sweet-corn α-glucosidase on a column (12 x 2.5 cm) of hydroxylapatite (Biogel HTP). Elution was carried out with a linear gradient of phosphate buffer (—— – —— – ——) of increasing molarity (5 mM to 200 mM over 1 litre), fractions of volume 6.0 ml being collected automatically. Residual protein was removed by further elution with 500 mM phosphate buffer pH 7.5. The distribution of protein in the fractions was determined by measurement of the absorbance at 280 nm (-------) in a 1 cm cell. The activity (————) was measured by the release of glucose from maltotriose at pH 5.1 and 30°C using glucose oxidase (9). The fractions under the three peaks were combined, viz. 82-88, 92-108 and 112-120.

peaks of activity (Fig. 1). The activities of the three fractions were 13.3, 17.0 and 11.5 International Units per mg of protein respectively, compared with the activity in the initial extract which was 0.059 units per mg, indicating purification of 225, 288 and 195 fold. The total recovery of activity was 52%. The activities were determined throughout using maltotriose as substrate in pH 5.1 citrate buffer at 30°C. When the specific activities were determined with maltose as substrate at 37°C in pH 3.0 citrate buffer, they were 64, 70 and 48 units per mg for the three fractions. This compares with specific activities of 7.7 for rat-liver α-glucosidase (3) and 34 for the bovine-liver enzyme (1) measured on maltose at 37°C and at the optimum pH values. All three fractions were shown to be free from α-amylase contamination by incubation with periodate-oxidized amylose and crystalline fungal gluco-amylase. We have previously shown that this is a sensitive method for detection of trace amounts of endo enzymes in exo enzyme preparations (10).

The pH/activity curve for the major fraction (peak 2) is shown in Fig. 2;

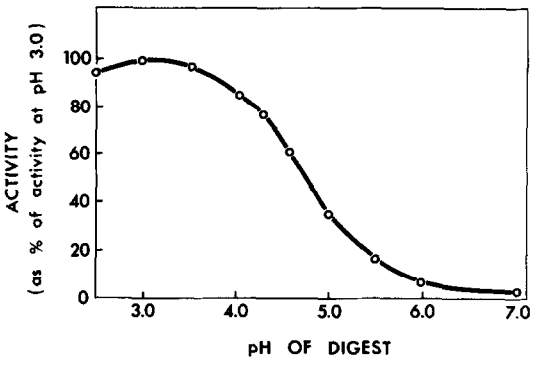

Fig. 2 pH/Activity curve for activity in peak 2 [fractions 92-108, (Fig.1)] from hydroxylapatite column. The enzyme fraction was dialysed against water, then diluted 100 times with human serum albumin solution (0.5 mg/ml). The activity digests contained maltotriose solution (10 mg/ml, 0.2 ml), 0.2 M citrate buffer of measured pH (0.2 ml), human serum albumin solution (5 mg/ml, 0.05 ml) and diluted enzyme solution (0.05 ml). After incubation at 30°C for 60 minutes the liberated glucose was determined using glucose oxidase (9).

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those for the other two fractions are closely similar. In all cases the optimum pH is in the region of 3.0 with a slight shoulder at pH 4.25. Above this value the activity drops sharply with increasing pH and all three fractions are essentially inactive above pH 7.0.

The stabilities of the three fractions at the optimum pH (3.0) were tested on incubation at 37°C. After various lengths of time at this pH and temperature the percentage activity remaining was measured using maltose as substrate. The results are shown in Fig. 3. It is clear that all fractions are unstable at this pH (at least in the absence of substrate), the half-life being approximately 3 hours under these conditions. Storage of the fractions at pH 7.5 and 2°C resulted in no detectable loss of activity over a period of two months.

Polyacrylamide disc-gel electrophoresis of the α-glucosidase fractions was carried out as described by Ornstein (11) in 7% gels using phosphate buffer pH 7.5. The gels were stained with Coomassie Blue and in all cases a single protein band was observed. The bands were of low electrophoretic mobility which was approximately the same for each. It is clear from the

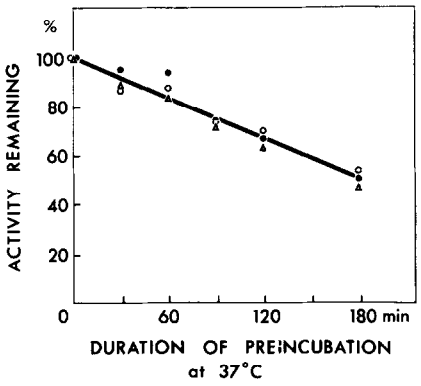

Fig. 3 Stability of sweet-corn α-glucosidase fractions at 37°C and optimum pH (3.0). Several 0.05 ml samples of each enzyme fraction (diluted 50-fold using a 0.5 mg/ml solution of human serum albumin) were preincubated at 37°C in the presence of 0.2 M citrate buffer pH 3.0 (0.2 ml) and human serum albumin solution (5 mg/ml, 0.05 ml). After incubation for various lengths of time the activity remaining was determined by addition of maltose solution (20 mg/ml, 0.2 ml) and incubation at 37°C. The amount of glucose liberated in 30 min was determined by removal of samples (100 µl) for glucose oxidase estimations, and the activity remaining after the various times of preincubation calculated as a percentage of the initial activity (● peak 1, fractions 82-88; ▲ peak 2, fractions 92-108; o peak 3, fractions 112-120).

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stabilities, the pH/activity curves and the disc-gel electrophoresis that there is little difference between the three forms of the enzyme. This is the second case we have come across where fractionation on hydroxylapatite has given fractions with closely similar properties. We have previously shown a similar phenomenon with sweet-corn R-enzyme (12). We presume that both fractionations are separations of isoenzyme forms.

All three enzyme fractions bring about hydrolysis with retention of configuration at the anomeric carbon atom of the liberated glucose molecule. This was shown by following the rate of oxidation, using glucose oxidase, of the glucose liberated from maltotriose as substrate by the action of the enzyme fractions before and after mutarotation by heating (13). Since mutarotation increased the rate of oxidation and glucose oxidase is specific for β-D-glucose, the liberated glucose must be in the α-configuration. This suggests that all three enzyme fractions are α-D-glucosidases (i.e. oligosaccharases), rather than exo-α-glucanases (14). Confirmation of this was obtained by

measuring the rate of release of glucose from various maltodextrin substrates. The rate was found to decrease as the chain-length increased as expected for glucosidases (14).

Sweet corn is unusual in that it contains glycogen as a reserve polysaccharide. By analogy with the mammalian systems where this type of enzyme is important for the degradation of glycogen (7), we suspected that these enzymes might be involved in the breakdown of the phytoglycogen. In order, therefore, to test this hypothesis we prepared extracts from mature seed of waxy maize and normal maize. The crude extracts were used directly, after dialysis and dilution, for determination of α-glucosidase activity using maltose as substrate. The pH/activity curves are shown in Fig. 4, and are similar to those for the sweet-corn α-glucosidase fractions. Our theory that this enzyme might be present in sweet corn specifically because of the presence of glycogen in this plant is therefore invalid since neither waxy maize nor normal maize contains any significant amount of this water-soluble polysaccharide. Figure 4 also shows the pH/activity curve for the maltose activity in an extract of "Malt Amylase PF" (Wallerstein). This enzyme also has a low optimum pH (3.75).

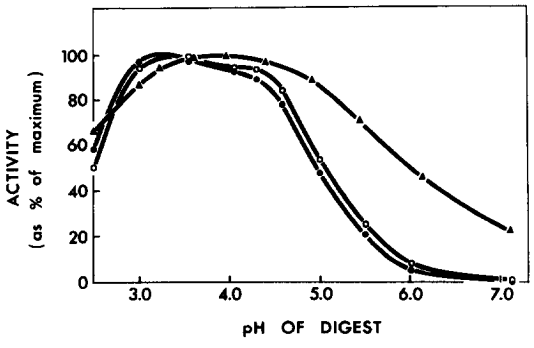

Fig. 4 pH/Activity curves for α-glucosidase activity in extracts of waxy maize (—●—●—), normal maize (—o—o—) and a commercial malt extract (—Δ—Δ—). The activity measurements were carried out as described in the legend to Fig. 2 except that maltose was used as substrate. The buffers used were citrate (up to pH 3.0) or citrate-phosphate (above pH 3.0).

It is therefore clear that mammalian tissues are not the only sources of acid α-glucosidase activity. We have shown that it is present in three mutants of *Zea mays* and a commercial malt amylase preparation. α-Glucosidase has also been reported in radish (15), alfalfa (16), carrots (17), tomatoes (17), barley malt (18), buckwheat (19), peas (20) and rice (21). Only the buckwheat enzyme has been extensively purified. In none of these cases, however, has an abnormally low optimum pH been reported.

The *in vivo* significance of this type of glucose-producing enzyme has not yet been clearly established but the suggestions have been made (20, 21) that it forms part of a non-phosphorolytic pathway for the breakdown of starch, its function being to bring about hydrolysis of the oligosaccharides produced by α and β-amylolysis. While it is clearly not the most favorable mechanism energetically, the possibility that a hydrolytic pathway is an important route for the degradation of starch must not be ruled out.

The type of enzyme which we have described is clearly different from other enzymes liberating glucose from maltodextrins, such as fungal glucoamylase, not only in pH optimum, which suggests that a considerably more acidic grouping is involved in the active site, but also insofar as hydrolysis takes place with retention of configuration rather than inversion. It is tempting to speculate on the possible differences in mechanism of action of the two types of enzyme, as has been done by Thoma (22) for α and β-amylases. However, we feel that further experiments are required before this can be done with any degree of accuracy. It is hoped that experiments in progress will lead to a clearer understanding of the detailed mechanism of hydrolysis by these enzymes.

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